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Eggshell Thinning and Pesticide Residues in Ospreys from the Lower Chesapeake Bay

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EGGSHELL THINNING AND PESTICIDE RESIDUES IN OSPREYS FROM THE LOWER CHESAPEAKE BAY

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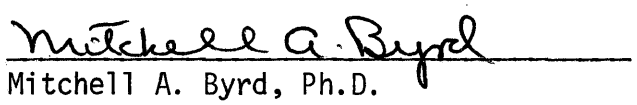
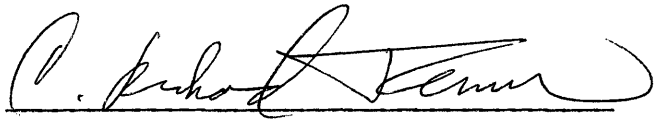


Mitchell A. Byrd, Ph.D.



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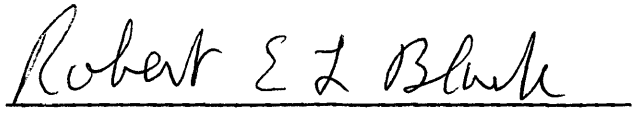

Robert E. L. Black, Ph.D.

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ABSTRACT

Eggs and eggshell fragments of the osprey, Pandion haliaetus, were collected during the summer of 1972 from nests in Tidewater Virginia and from the Eastern Shore of Virginia. In all, 163 egg shell and shell fragments were examined for possible alterations in shell thickness and weight when compared to eggs in museum collections collected prior to 1947. Eighty-five of the eggs opened were also analyzed in order to quantify the chlorinated pesticides and other chlorinated pollutants. Most of the eggs collected were those which were presumed infertile or addled. The procedure for analysis was a modified procedure from the Patuxent Wildlife Research Center.

Nearly all of the eggshells were thinner than those collected prior to 1947. An 18.6% mean decrease in eggshell thickness was found for the entire study area. Eggshell fragments from broken eggs were usually significantly thinner ($p<0.05$) than eggs with some degree of development. Residues of pp' DDE, pp' DDT, pp' DDD, dieldrin, Aroclor 1254, heptachlor epoxide, and dichlorobenzophenone were found in most eggs. Residues of endrin and lindane were only occasionally found.

No relationships were found between concentrations of any of the pesticide residues and shell thinning. However, inability to establish any relationship could most likely be due to a bias in egg collection. Residues of pp' DDE were found to be significantly higher ($p<0.01$) in eggs from unsuccessful nests when compared to successful nests which hatched at least one chick.

EGGSHELL THINNING AND PESTICIDE RESIDUES IN OSPREYS FROM THE LOWER CHESAPEAKE BAY

INTRODUCTION

Since the World War II industrial boom, populations of many raptor species have markedly declined. In Europe, decreases have been especially noted in the Peregrine falcon, Falco peregrinus and the Golden eagle, Aquila chrysaetos (Ratcliffe, 1967). Similar trends in North America have been well documented for populations of the Peregrine falcon, Prairie falcon, Falco mexicanus, Bald eagle, Haliaeetus leucocephalus, and the osprey, Pandion haliaeetus(Berger, et al., 1969; Cade, et al., 1971; Fyfe, et al., 1969; Sprunt, 1969; Peterson, 1969a).

The marked declines in several osprey populations have resulted in considerable research on this species throughout the continent. However, the most intensely studied areas are the more industrialized centers of the east coast of the United States. It is in these areas that the more drastic population declines have occurred. (Spitzer, 1972, unpublished data). For example, several northeast populations show a 13 - 14% annual rate of decline whereas populations of the Chesapeake Bay show only a 2 - 3% annual rate of decline (Henny and Ogden, 1970).

A chief factor responsible for the population declines is an increased incidence of egg breakage (Ratcliffe, 1967; Ames, 1966; Anderson and Hickey, 1970). This is, in turn, due to a decrease in shell thickness which has been linked by many biologists to the presence of p'p' DDE in the eggs. (Wiemeyer and Porter, 1970; Blus, et al., 1971; Cade, et al., 1971). This compound is the major metabolite of the once widely used pesticide, DDT.

Analysis of eggs and tissues show a wide variety of other chlorinated pesticides and large amounts of recent industrial pollutants, polychlorinated biphenyls (PCB) (Risebrough, et al., 1968; Peakall and Lincer, 1970).

For the past five years, Dr. Mitchell A. Byrd, College of William and Mary, has studied the osprey population of the lower Chesapeake Bay and Tidewater Virginia. The Chesapeake Bay osprey population is probably the largest existing population in the world as it has not yet shown the drastic decline characteristic of the northeast. Under the direction of Dr. Byrd, Robert Kennedy (Kennedy, 1971) initiated the osprey studies at William and Mary. Mr. Kennedy has since graduated and this research has been continued by Gary Seek for the past two years (Seek, 1974). Their study area included all of the Virginia shore of the Chesapeake Bay and its Virginia estuaries. Coverage of the Potomac River has been limited to the Virginia shore from Currioman Bay to the east. Both the Atlantic Ocean side and Chesapeake Bay side of the Eastern Shore of Virginia are covered. The reader is referred to Seek (1974) for a detailed description of the study area.

The osprey research program at William and Mary is primarily an intensive study of the population dynamics of the species. The survey begins early in the spring by locating all osprey nests. These nests are closely observed during the breeding season to ascertain the number of eggs laid, the number of eggs hatched, and the number of fledglings produced from each nest. Most of the young are color banded for later identification. More recently several techniques have been investigated which may increase the productivity of the population by clutch manipulation. As a by-product of the population survey, eggs and eggshell

fragments are collected whenever possible.

The presence of a large, relatively stable osprey population makes possible a wide range of research activities. In view of the population declines of the osprey and the large amount of industrial pollution in other areas, this research was concerned with an analysis of osprey eggs for the presence of a major group of industrial pollutants, the chlorinated hydrocarbon insecticides. The intent was to collect as many eggshell fragments and entire eggs as possible without damaging the existing population and to run a chemical analysis on these samples. Eggshells were to be examined for any changes which would indicate weakening or thinning. Contents were analyzed with an adapted method from Patuxent Wildlife Research Center in order to determine the chlorinated hydrocarbon residues present. Hopefully, the relationship between eggshell changes and pesticide levels would help to explain why the annual rate of decline for the Chesapeake Bay population is considerably less than that of osprey populations along the northeast coast.

LITERATURE REVIEW

The osprey, Pandion haliaetus, is the only species in the family, Pandionidae. Since its distribution is cosmopolitan throughout the northern hemisphere, it has been the subject of many studies on various aspects of its biology. While several studies have been made in Europe and Scandinavia (Siewert, 1941; Moll, 1962; Moll, 1969; Odsjo, 1971), the bulk of published research is from the United States. Chief concern has been given to population studies on the East Coast. However, many other studies of osprey populations have been made across the United States (lower California, Kenyon, 1947; Minnesota, Ingrim, 1966; Michigan, Ingrim, 1966, Postupalsky, 1966, 1968a, 1968b, 1969; Wisconsin, Ingrim, 1966, Berger and Mueller, 1969; Idaho, Koplin, et al., 1969). Many other studies on osprey biology are in press in the Proceedings of the First North American Osprey Conference which was held at the College of William and Mary in February, 1972.

A survey of several United States osprey populations by Henny and Ogden, (1970) shows a 13 - 14% decrease annually, whereas Chesapeake Bay populations show only a 2 - 3% annual decline. In order to maintain population stability, Henny and Wight (1969) and Henny and Ogden (1970) calculated that populations must produce between 122 and 130 young per 100 nests each year (1.22-1.30 young per nest).

Osprey populations along the northeast coast probably show the most significant declines. For example, Gardiner's Island at the southern

end of Long Island, New York, was a stronghold for ospreys for many years. Leroy Wilcox, who has banded ospreys for more than thirty years, estimated 300 nests in 1945 (Peterson, 1969a). In 1965 there were only 26 nests (Peterson, 1969a) and in 1966 only 3 nests were found (Spitzer, 1972). Fortunately, in 1970, Spitzer found 38 nests in this colony.

On eastern Long Island, New York, there were approximately 500 nests in 1941. In 1965, only 25 to 30 nests produced young (Peterson, 1969b). Nearby Orient State Park formerly had 35 nests, but only 1 nest in 1965 (Peterson, 1969b). In 1968 and 1969 there were 8 nests in the Park which produced no young. In 1969, there were 9 nests which produced 2 young (Spitzer, 1972).

Another well-studied osprey population which has shown considerable decline is found on the Connecticut River. In 1938, the area of the Connecticut River contained approximately 200 nests (Peterson, 1969b). In 1968, Peterson found the remaining 10 nests produced only one young. Peterson estimates that production in this colony is only 1/8 that of normal levels which results in a 33% annual rate of decline in this population. Henny and Ogden (1970) arrived at a more conservative figure of 13 - 14% annual decline which is similar to the New York populations. Productivity in this colony is very low with only 0.29 birds per active nest being fledged (Ames, 1966).

Similar population declines have occurred in Maine where an 80% decrease has occurred in the last 10 years (Peterson, 1969b). Productivity of 13 nests in Maine was also low (0.37 birds per active nest) (Kury, 1966).

In Rhode Island there has been a 60% decline in the osprey population in the past 20 years. (Peterson, 1969b). Twenty-five percent of

this decline occurred from 1961-1969. A poor fledging rate of 0.37 young per active nest was found for this same period (Spitzer, 1970).

Three large populations in New Jersey have shown similar declines. In one study area, 25 nests produced about 50 young in 1930. In 1963, the same area contained 7 non-productive nests (Schmid, 1966). In 1929, there were 30 nests around Avalon, New Jersey and unlike other areas there were still 29 nests in 1963 with a productivity of 1.6 birds per active nest (Schmid, 1966). Even though the number of nests remained relatively stable, the productivity decreased to 0.28 birds per active nest in 1968 (Spitzer, 1970).

In southern New Jersey, there has been a 95% decrease in the population since 1960 (Peterson, 1969b). Herbert Mills reported that in 1950 there were 253 nests in southern New Jersey most of which were around Cape May. In 1969 there were no nests in the Cape May area (Peterson, 1969).

Unlike the rampant declines in the northern populations, the osprey populations of the Chesapeake Bay area have remained relatively stable. For example, Schmid (1966) found that the nests on Tilghman Island in the bay were still producing 1.8 birds per active nest.

Along the middle of the bay in eastern Maryland, Jan Reese has been studying the osprey around St. Michaels, Maryland and the Choptank River since 1963 (Reese, 1968, 1969, 1970, 1972, 1973). He has not found any severe population declines but has found gradual declines in some areas.

In 1963, his Maryland population showed a productivity of 0.64 birds per active accessible nest. For the same area, Reese found 0.81 and 1.16 fledglings per active accessible nest for 1964 and 1965 respectively (Reese, 1970). From these productivity figures, Henny and Ogden (1970)

computed an annual rate of decline of 2 - 3% a year for this area. Productivity exceeded this level in 1970, 1972, and especially in 1973 when 1.43 young per active accessible nest was found. Although some of Reese's computations are different from those in many studies, the population trends of the Maryland population are much better when compared to the northeastern populations. Perhaps this populations will increase if the high fledging rates continue.

Other osprey populations in the Chesapeake Bay do not show such a high degree of success. The ospreys of the eastern Potomac River on the Maryland shore and Nomini Bay on the Virginia shore have been studied by several workers over the last 10 years (Wiemeyer, 1971). In more recent years, the study area has been continued by Stan Wiemeyer, Patuxent Wildlife Research Center in Laurel, Maryland. From 1963 to 1968, productivity was below the level necessary to maintain a stable population (Wiemeyer, 1971).

When compared to productivity of the area in 1963 and to the population data of Jan Reese from across the bay, productivity of Potomac River ospreys has taken a considerable drop. Wiemeyer (1970, 1972) found a productivity of 0.70 fledglings per accessible nest in 1970 and 0.56 fledglings per accessible nest in 1972. Despite the low productivity, recent drastic reductions in the breeding populations do not seem to have occurred.

Population changes appear to have occurred in the study area considered by Seek (1974) when data are compared with earlier published accounts. For example, Tyrell (1936) found 46 nests in the Smith Point area of the Potomac River in 1936. The same area

in 1972 contained 20 nests, Seek (1974). Field notes from an egg collector, F.M. Jones, cites osprey nests on Bay Tree Neck, below the York River in 1935 (unpublished field notes). Jones describes the 28 occupied osprey nests he found on this small marsh. In 1974 the same area had no nests (unpublished information, M.A. Byrd).

Most of the above Chesapeake Bay population was studied by Robert Kennedy in 1970 and 1971, Kennedy (1972). He found production to be below the level necessary for a stable population. He found 0.96 fledglings per active nest in 1970 and 0.69 fledglings per active nest in 1971. In 1934, Tyrell found that there were 2.2 - 2.5 birds produced per productive nest in the Smith Point area. In 1970 and 1971 the number of birds per productive nest was 1.88 and 1.84 respectively. Therefore, there has been a decrease in productivity. A further decrease in productivity was found in 1972 with 1.63 birds per productive nest but an increase in productivity per productive nest (1.91) was found in 1973, Seek (1974). Even though productivity data are below the levels suggested by Henny and Ogden (1970) for population stability, field surveys show that the population has remained relatively stable during the five years of study.

Examination of osprey population studies brings forth many factors which may be responsible for decline. However, in most studies decline cannot be related to any one factor but perhaps an interaction of several factors.

FACTORS POSTULATED FOR OSPREY DECLINE

Early studies with declining osprey populations suggested perhaps that predation played a major role in reducing productivity. Leroy Wilcox believed one factor contributing to the decline of the osprey on Gardiner's Island was the expansion of a nearby herring gull, Larus argentatus, colony (Peterson, 1969b). This factor fails to take into account other species on Gardiner's Island away from the gull colony which are also declining. The black-crowned night heron, Nycticorax nycticorax, has shown a 90% decrease in the last 10 years on Gardiner's Island (Peterson, 1969b). This assumption also does not take into account the areas where population declines have occurred and no gull colonies are found. Ames and Mersereau (1964) did not think gull and other avian predation was a major factor because of the aggressive nature of ospreys. Since they suspected raccoon, Procyon lotor, predation, they erected predator-proof nesting platforms, but found little change in productivity on the platform nests. Similar results with platform nests have been found by other workers which seems to indicate that mammalian predation is not a major factor, (Kennedy, 1971; Seek 1974). Reese suspects that mammalian predation may be an important cause of egg loss. Raccoons were involved in 20 incidents of egg breakage from 1963-1970 (Reese, 1970). Fernandez also believes mammalian predation is a major cause of egg loss of his Massachusetts colony which is comprised largely of nests built in trees (Reese, 1970). However, predation in Reese's area was severely reduced on offshore nesting structures and could not account for egg loss on these

nests (Reese, 1970).

Hatchling loss is more difficult to detect due to absence of eggshells which may be possible clues. Reese (1970) found a total of 58 hatchlings disappearing between the years of 1963 and 1968, but evidence of predation accounted for only four young. Seek (1974) found that 18.2% of the hatchlings disappeared in 1972 and 8.4% were lost in 1973. Even though mammalian predation may account for sporadic egg and hatchling loss, it is unlikely that it is a major contributing factor to the decline of osprey populations.

Human interference has been an important factor in osprey declines. For example, the past policy of the United States Coast Guard along the Atlantic Coast was to remove osprey nests from navigational aids (Stickel, et al., 1965). This action was responsible for destruction of 68 eggs and 12 fledglings in Reese's study area from 1963-1968 (Reese, 1970). In our study area Kennedy (1971) and Seek (1974) believe some missing nests are the direct result of Coast Guard activity. In 1972, a Coast Guard directive was issued forbidding removal of any osprey nests on navigational aids in the Fifth Coast Guard District without first consulting Dr. Mitchell Byrd at the College of William and Mary. Other sporadic incidents of nest destruction, removal of young from nests, and shooting occur every season (Reese, 1970; Kennedy, 1971; Seek, 1974), but these are not believed to be a major factor in population decline.

A more nebulous effect of man would be his destruction of the shorelines where many ospreys nest. Human activity was thought to be related to osprey declines (Schmid, 1966; Ames & Mersereau, 1964; and Reese 1968, 1970, 1973). Increased building along estuarine shorelines has eliminated many nest sites and made the remaining nest sites intolerable for ospreys.

Reese (1973) blames 11 of the nest failures in his study area on the recent increase of sport boating enthusiasts. Failure of several nests on the Chickahominy River in Virginia and other places in the William and Mary study area may be related to the same factor (Seek, 1974).

Restrictions of other water activities may aid osprey production. Reese (1973) mentions one major reason for increased production in his study area for 1973 would be the restrictions on clam fishermen. Because fewer clam fishermen were allowed on the water, the sediment was not stirred up by the clamming process. This restriction resulted in clear water for osprey fishing.

Despite the many disturbances by man, ospreys are relatively tolerant of man and may build nests in areas of moderate human activity early in the season, some of which are abandoned as the season progresses. To test the effects of human encroachment, Ames and Mersereau (1964) compared nests in the Connecticut River Basin to see if remoteness of nests had any effect on productivity. They found no difference in the productivity of remote nests as compared to nests in close proximity to human habitation.

Changes in the fishing industry are believed by several biologists to be a major factor in the osprey decline. Limited use of the once popular pound net along the southern New Jersey shore possibly has eliminated many easily caught fish from the resident ospreys (Schmid, 1966). Recent migration changes of several anadromous fishes could also be an important factor. Schmid (1966) feels there are not as many fish entering the estuaries and, as a result, ospreys are not attracted to these areas for breeding. Reese (1973) does not feel that food is a limiting

factor because of the high osprey productivity in his area.

Osprey productivity may also be influenced by climatic factors. Several studies discuss the effect of heat, lightning, hard rains, and hurricanes (Ames and Mersereau, 1964; Tyrell, 1936; Reese, 1970, 1973; Kennedy, 1971; Seek, 1974). Examination of climatic factors shows that none are consistent enough to explain the declines many osprey populations have experienced in the last two decades. However, climatic factors may interact with other factors to cause fluctuations in productivity from year to year (Reese, 1970; Reese, 1973; Kennedy, 1971; Seek, 1974).

Many osprey populations demonstrate declines which cannot be explained solely on the basis of any of the above factors or adult mortality rates (Ames and Mersereau, 1964; Peterson, 1969b; Ratcliffe, 1970). Most of these populations also are less productive than the figures indicated by Henny and Ogden (1970) necessary to maintain a stable population. In general, decreased productivity is most likely the reason for osprey declines since survival rates for first year birds and adults show little change between 1926-1961 (Henny and Wight, 1969).

Failure of the eggs to hatch has been the chief factor given by many workers as the major reason for osprey declines (Ames and Mersereau, 1964; Ames, 1966; Peterson, 1969a, 1969b; Reese, 1970, 1971; Kennedy, 1971; Seek, 1974). Examination of data from most of the populations show a low hatching rate, for example, from 1961-1963 the hatching rate averaged 12% in the ailing Connecticut River population (Reese, 1970). Hatching success has fluctuated greatly in the Massachusetts population and may account for the unstable productivity. The hatching success for this colony showed no trends and averaged 33% for the period between 1965-1970.

Reese found the hatching rate in the upper Chesapeake Bay to be in the low 40th percentile range until 1972 when it reached 57% and 54% in 1973. Wiemeyer (1972, unpublished data) found a 17% hatching success in his study area in 1971. Kennedy found a hatching success of 38% in 1970 and a 37% hatching rate in 1971. The same population showed an increased hatching success of 43.1% in 1972 and 51.9% in 1973 in nests with known clutch size (Seek, 1974).

The role of egg failure in productivity was further demonstrated in an experiment in which eggs were switched between Maryland and Connecticut during 1968-1969 (Peterson, 1969a; Spitzer, 1970; Peakall, 1970a). The eggs transported from Maryland showed a hatching success of 44% in Connecticut nests while the eggs from Connecticut ospreys had a hatching success of only 12% in Maryland nests. This experiment demonstrated that hatching failure is not caused by predation or human disturbance, but by some factor intrinsic to the egg.

Of the eggs that do not hatch, the greatest percentage of eggs would be those which disappear between visits whether due to breakage, predation or weather. Of 745 eggs not hatching in Reese's study area between 1963-1968, 403 or 54% disappeared between visits (Reese, 1970). Kennedy found that 58% of the eggs not hatching disappeared between visits (Kennedy, 1971). Seek found in the same area a 57.3% and 47.8% disappearance for 1972 and 1973, respectively.

Many of the eggs were found with breakage such as cracks or punctures or were found as only shell remains. Eggs with hairline cracks were shown in one study to reduce the hatchability of the egg by 50% (Heath, et al., 1969). Reese (1970) found between 1963-1969 that 11% of the 745 eggs which did not hatch were found broken in the nest. However, in 1973 half of the

eggs which did not hatch were broken in the nest (Reese, 1973). Kennedy (1971) found only 4.5% of the unhatched eggs were broken while Seek found 10.9% breakage of unhatched eggs in 1972 and 8.3% in 1973 (Seek, 1974). Breakage might explain the larger number of eggs which disappear between visits. Fragments of broken eggs may be removed or eaten by the adults as is done by other raptorial species (Ratcliffe, 1958; Ratcliffe, 1970; Porter and Wiemeyer, 1969).

Egg breakage could indicate some structural defect in the eggshell which would make it weaker. To investigate this question, Hickey and Anderson (1968) compared a sample of 229 osprey eggs collected from eastern North America prior to 1947, with recently collected eggs from New Jersey. They found the eggs from New Jersey were 25% lighter than those collected before 1947. However, eggshells from the more stable populations of Maryland and Virginia show only a 2.0 to 2.8% decrease in shell weight. A more recent study utilizing 365 eggs collected prior to 1947 shows that eggs recently collected from Maryland, New Jersey and Connecticut are 21% thinner than the pre-1947 eggs (Anderson and Hickey, 1970a). One European study also found the onset of shell thinning in osprey eggs to be around 1947-1949 in Sweden and Finland (Odsjo, 1971, reviewed in Cooke, 1973). In this study, eggshell thickness has continually declined since 1949.

Many other scientists along the east coast have reported shell thinning in osprey eggs. Spitzer (1972) reports a 22% shell thinning in the ailing populations of Connecticut and New Jersey for 1970. The population along the Westport River in Massachusetts shows an 11% shell thinning and an 18% shell thinning was found for Gardiner's Island in 1970 (Spitzer, 1970). Wiemeyer (1972 unpublished data) found a 12% shell thinning for 1968-1969 and a 13% thinning in 1970-1971 for his Maryland study area.

The lower Chesapeake Bay showed 15% eggshell thinning in 1970 and 27% shell thinning in 1971 (Kennedy, 1971). Despite the population stability of the Florida Bay osprey colony, a 13% decrease in thickness index was found (Anderson and Hickey, 1970a).

Reduction in shell thickness and other shell parameters was first observed by a British scientist (Ratcliffe, 1967) who thought shell thinning was a factor responsible for declining populations of the peregrine falcon and golden eagle. Ratcliffe also observed the peregrine falcons eating their own eggs (Ratcliffe, 1958; Lockie and Ratcliffe, 1964). To test the hypothesis that recent raptor eggs were inherently weaker, Ratcliffe compared the eggs of three European species, the peregrine falcon, Falco peregrinus, golden eagle, Aquila chrysaetos, and European sparrow hawk, Accipiter nisus, with a large sample of eggs from museum and private collections. In order to evaluate and compare intact museum shells, Ratcliffe devised the Thickness Index (T.I.) formula. The formula makes use of the length (l) and breadth (b) in cm of the egg and the weight (w) in mg such that: T.I.= w/lxb (Ratcliffe, 1967). Using the thickness index, Ratcliffe found a highly significant (p 0.001) decrease in the thickness index for all species. Since 1946 and 1947 marked the onset of sudden decreases in the shell thickness of many species, only those eggs collected before 1947 were used as a reference source. Ratcliffe correlated the data with the onset of extensive use of chlorinated insecticides which he believed responsible for the changes in eggshell thickness.

In the United States, several raptor species including the bald eagle, peregrine falcon, osprey and sharp-shinned hawk, Accipiter striatus, have shown similar decreases in eggshell thickness (Hickey and Anderson, 1968; Anderson and Hickey, 1970; Snyder, et al., 1973). However, several raptor

species including the red-tailed hawk, Buteo jamaicensis, golden eagle, Aquila chrysaetos, and great horned owl, Bubo virginianus, do not show eggshell thinning. Further work by Anderson and Hickey (1972) examines population declines and shell parameter changes in 25 species of upper tropic level birds. Varying degrees of thinning were found in raptorial species, but fish-eating birds show the greatest degrees of shell thinning (Hickey and Anderson, 1968; Faber and Hickey, 1973). Brown pelicans show the most shell thinning of any species (50%) in the Anacapa Island colony in California (Risebrough, et al., 1970, 1971; Keith, et al., 1970). Many other species also exhibit eggshell thinning in nature and are reviewed in an extensive paper on eggshell thinning by Cooke (1973).

Following the determination of changes in shell parameters, many studies investigated the levels of pesticides in tissues and eggs of wild birds. Examination of a broad spectrum of avian species shows the greatest effects and residues were found in the higher tropic levels, especially the piscivorous species (Anderson and Hickey, 1970; Faber and Hickey, 1973).

Analysis of bald eagle eggs from three populations in the United States show some interesting trends (Krantz, et al., 1970). Eggs from non-productive nests in Maine contained 21-76 parts per million (ppm) DDE and 1.41 ppm Dieldrin. In contrast, the productive nests of Florida and Wisconsin contained an average of 10.72 ppm of DDE with 0.21 ppm Dieldrin and 4.76 ppm DDE with 0.37 ppm Dieldrin, respectively. Analysis of two residues were in excess of lethal doses (Riechel, et al., 1969).

Peregrine falcon eggs also have been analyzed and show significant levels of DDE (Cade, et al., 1971). Egg analysis of several Alaskan populations of peregrine falcons revealed up to 3,130 ppm DDE, lipid basis.

This study also found DDE to be significantly related to the decrease in the thickness index. Analysis of adult peregrine tissues showed that in Alaskan birds, adults contained 23 times the pesticides as a young bird ready to migrate.

Brown pelicans, Pelecanus occidentalis, which have shown 34% -53% in shell thinning in some colonies (Risebrough, et al., 1970; Keith, et al., 1970) have also been the subject of pesticide analysis. Residues from eggs from the Anacapa Colony showed DDE levels as high as 2,500 ppm in egg lipids (Risebrough, et al., 1970). Several east coast pelican eggs were analyzed and levels of DDE averaged 71 ppm on a wet weight basis (Blus, et al., 1971). This study also showed a significant correlation between increased levels of DDE and increased shell thinning.

In a recent study of North American accipiter hawks, the sharp-shinned hawk, Accipiter striatus shows the greatest levels of DDE of the three North American species (Snyder, et al., 1973). A significant correlation ($p<0.05$ was also found between levels of DDE and shell thinning.

The osprey has also been studied for chlorinated pesticides. Stickel, et al., (1965) found 3.4 ppm DDE wet weight in 41 Maryland eggs. Twenty-one eggs from the less productive Connecticut colony were found to contain more DDE, 6.5 ppm wet weight. Another early study analyzed eggs from several Northeastern colonies and also found that the levels of DDE in the northeast (5.1 ug/ml) were greater than those of the Maryland population (2.3 ug/ml) (Ames, 1966).

A more recent analysis of osprey eggs also shows greater pollutant levels in the Northeastern birds when compared to a healthy population in Baja, California (Spitzer, 1972). Increased levels of pesticides have been cited as the major reason for low hatching of the eggs transported

from Connecticut and placed in Maryland nests (Peakall, 1970a). Analysis of seventeen 1971 Potomac River osprey eggs shows an average of 3.5 ppm of DDE wet weight and 0.23 ppm of Dieldrin (Wiemeyer, 1972, unpublished date).

To determine further the relationship between shell-thinning and pesticides, a large number of studies were conducted in which different experimental species were fed varying concentrations of pesticides. One of the early studies involved feeding of p'p' DDE or p'p' DDT to female mallard ducks (Heath, et al., 1969). Feeding 40 ppm in the diet for one to two years produced up to a 13% decrease in the shell thickness in the higher dosed groups. Hatchlings from the higher dosed group also showed a 35% decrease in survival.

Most feeding studies show decreases in shell thickness when DDT, DDE, or in some cases, Dieldrin, are fed to experimental animals (Cooke, 1973). Many studies using chicken, Gallus domesticus, and similar gallinaceous species do not show these results. The chicken is, however, an atypical species as it may tend to be resistant to DDT (Risebrough, et al., 1970; Cooke, 1973). Porter and Wiemeyer (1969, 1970, 1972) found the American Kestrel, Falco sparverius, to be a species adaptive to captivity for purpose of feeding studies. This species is more comparable to those raptors studied in nature since it is a falconiform bird and would normally feed at a similar trophic level. Feeding controlled levels of DDE in the diet produced eggshells 10% (sig. $p<0.001$) thinner after more than one year of treatment (Wiemeyer and Porter, 1970). Feeding a mixture of DDT and Dieldrin caused 8-10% thinning of eggshell (Porter and Wiemeyer, 1969). The effect of feeding was much more apparent in the first year offspring from the DDT and Dieldrin fed birds since the offspring showed a 15-17% decrease in

shell thickness (Porter and Wiemeyer, 1969). Porter and Wiemeyer also found an increase in egg breakage and egg disappearance and a lowered productivity. Both of these feeding studies point to possible linkages between presence of DDT, DDE, and or Dieldrin and shell thinning.

Changes in eggshell thickness may result from factors other than pesticides. Dietary deficiencies, other chemicals and age are all related to shell thinning (Taylor and Stringer, 1965; Romanoff and Romanoff, 1949; Simkiss, 1967; Lillie, et al., 1973). Environmental temperature and humidity were also found to affect shell thickness (Elbousky, et al., 1968). Sequence of the egg in the clutch also affects thickness of eggshells (Romanoff and Romanoff, 1949). Other environmental factors and geographical variation also affect shell thickness in some species (Anderson and Hickey, 1970; Blus, et al., 1970). Considerable attention has been given recently to development as it relates to shell thinning. Developing embryos also decrease shell thickness by utilization of eggshell calcium (Simkiss, 1967, 157-197; Vanderstoep and Richards, 1971; Anderson and Hickey, 1970; Kreitzer, 1972, 1973; Rothstein, 1972).

Despite the many variables mentioned which may affect shell thickness pesticides of the DDT group have consistently been linked to shell thinning in laboratory feeding studies and in many studies in nature. The actual mechanism of shell thinning is currently a debated topic. Certainly, the mechanism in some way inhibits the transfer of calcium ions to the shell gland of the female bird, but the processes which interfere with the transfer are difficult to interpret. The reader is again referred to an excellent review paper by Cooke (1973) which gives an unbiased view into the current research of mechanisms of shell thinning.

MATERIALS AND METHODS

Collection of Shells and Eggs

Data for thickness measurements are taken from two sources, eggshell fragments and eggshells from entire (intact) eggs. Eggshell fragments were collected from the nest or from the ground at the base of the nest. Eggshell fragments from broken eggs are identified by the presence of dried egg yolk, various types of dirt or the shape of the shell fragments. If the allantoic sac and/or traces of the vascularized chorion were present, the shell fragments were considered to be from an egg from which a chick had hatched. Shell fragments from hatched chicks were usually larger than fragments from broken eggs. Fragments were returned to the laboratory where they were washed to remove dirt, egg yolks and fecal material. Care was taken not to remove the two eggshell membranes which line the inside of the shell. After cleaning, each fragment was placed in a plastic bag with a tag giving: identification number, collection site, and nature of the fragment (i.e., from hatched or broken egg). Identification numbers followed the system of Robert Kennedy (1971) and Gary Seek (1974) in which a prefix identifies the 7-1/2 minute quadrangle, United States Geological Survey Map, on which the nest site was located. This prefix is followed by the nest identification number. Following the nest number is a clutch designation number which serves to differentiate eggs within the same clutch.

Entire eggs were also collected for thickness measurements and pesticide analysis. Most of the entire eggs collected were found addled in

the nest. Several criteria were used to determine whether an egg was addled and would not hatch. First, rotation of addled eggs produces a splashing noise which is uncharacteristic of viable eggs. Addled eggs are also often lightweight (30g.) and may have the contents displaced to one end. Any eggs found in a nest in which there were chicks twelve days old or older were collected, as it was presumed they would not hatch. These collecting procedures eliminated from field collections all viable eggs with embryos, hence the interpretation of data is difficult.

Another source of entire eggs was a group which were removed from nests and were incubated in the laboratory, but failed to hatch. In April, 1972, 63 fresh eggs were brought into the laboratory with the hope of hatching young to be placed in foster nests. However, due to improper incubation, only 13 of the eggs hatched. Two eggs were pipped, but the chicks died before hatching. The remaining 48 unhatched eggs were included for analysis. These eggs were termed manipulated since they were removed from the field and subjected to artificial incubation. Since these eggs were randomly collected, they probably included many eggs which would have hatched in the field. Non-manipulated eggs refer to the remainder of the collected entire eggs.

All entire eggs, including manipulated eggs returned to the laboratory, were weighed to the nearest 0.01g. on a top-loading Satorius Balance. Eggs were measured to the nearest 0.01mm. for breadth and length using a Mitutoyo Dial Micrometer from Laboratory Supplies Company, Inc., 29 Jefry Lane, Hicksville, New York. Measurements were taken at the greatest breadth at the equator, and the greatest length on the longitudinal axis.

Eggs were also measured for volume (nearest 0.1 ml.) by weighing the amount of water displaced by the egg when immersed in a full beaker.

Eggs which were cracked or punctured were not immersed for volume determinations. Instead, approximate volume was obtained by a formula devised by Stickel, et al., (1973) ($v=0.48\ LB^2 + 3.69$) in which v=volume, L=length of egg in cm and B-breadth of egg in cm. By using the egg volumes and egg weights, egg contents were adjusted to a fresh weight basis. Since many eggs were addled or exposed to prolonged incubation or weather, considerable drying of egg contents may have occurred. Unless eggs can be adjusted to a fresh weight basis, bias may occur since the loss of water from the egg would result in more concentrated residues in the remaining egg contents. Adjustment to a fresh weight basis is made by multiplying the volume of each egg by the number of grams per milliliter of volume for a fresh egg. Sixteen fresh osprey eggs were measured for volume and weighed. The resulting relationship was found to be 1 gram of egg contents for each milliliter of volume (1g/1ml).

When enough material was present, 20g aliquots from the entire eggs were used because this was the largest aliquot for optimal handling and for satisfactory pesticide recovery (Krantz, et al., 1970). However, the fresh weight basis of the aliquots could often be much greater. For example, if an egg measured 60 ml in volume, it should weigh 60 grams (60ml X 1g/1ml). But, if this egg weighed 40 grams, the correction factor would be 60g/40g = 1.5. If a 20 gram (actual weight) aliquot was removed from this egg and adjusted to a fresh weight basis, a 30 gram (fresh weight) aliquot would result since 20g X 1.5 = 30g. All pesticide residues would then be expressed on a fresh weight basis for greater accuracy. Because of unavailability of contents material, not all aliquots were as large as 20 grams, but all aliquots were adjusted to a fresh weight basis.

Analysis Procedure

Entire eggs were stored for 2 to 4 weeks in a refrigerator at 45°C until they could be prepared for analysis. Upon removal from refrigeration, eggs were reweighed to adjust for moisture loss. Each egg was washed in warm water and rinsed three times in acetone to remove possible contaminants. Eggs were opened by a Dremel Motor Tool (a high speed drill) which cut a groove in the eggshell without damaging the shell membranes. After making the first 1/4 inch of the groove, the membranes were punctured in order to release gas which could blow the egg open. The groove was then continued completely around the equator of the shell. The halves of the shell were separated over a tared beaker by cutting through the shell membranes around the equator with a scalpel. Contents were emptied into the tared beaker and set aside for future analysis.

Eggshells were washed with special care so that the two internal shell membranes were not removed. Shells were placed on blotting paper and were allowed to dry for several weeks. Dried eggshells were weighed to the nearest 0.01g. Careful weighing before and after opening 16 eggs revealed that an average of 0.09 grams is lost by making the groove in the shell. This value was added to all subsequent weights as a correction factor. Thickness of each shell plus its membranes was measured to the nearest 0.001mm. at four points around its equator (Porter and Wiemeyer, 1969) with a MG Micrometer (No. 1000mm. from Laboratory Supplies, Inc.) equipped with a Neo-Ball adaptor from Neo-Ball Co., 669 MacArthur Avenue, Redwood City California. The adaptor is necessary for accurate measurements without variations due to shell curvature. The four values were averaged in order to obtain the mean shell thickness. Thickness was measured on eggshell fragments which had both shell membranes. Attempts

were made to choose shell fragments from the equator region of the shell since the poles of the eggshell are thicker.

All eggshells and fragments were then remeasured in area where the shell membranes had been removed to see if there were any differences which the presence of the membranes may have obscured. Shells and fragments were then stored in separate plastic bags with identification labels in a specimen case.

Contents of the entire eggs were weighed to the nearest 0.01 gram and the degree of embryonic development was noted. Eggs showing development were aged by weeks on the basis of embryo size. Contents were homogenized in a Waring stainless steel commercial blender (Model 5010). Egg contents were then stored in prewashed 30 ml culture tubes which had been rinsed three times with acetone.

Analysis procedure basically followed the procedure of the Patuxent Wildlife Research Center (Mulhern, et al., 1970). However, substitutions of similar solvents were made due to the unavailability of desired solvents (specific substitutions will be mentioned later). All solvents, with the exception of acetone, were pesticide grade and distilled in glass (Burdick and Jackson Laboratories, Inc., Muskegon, Michigan, 49442). Acetone, obtained from Fisher Chemicals (7722 Fenton Street, Silver Spring, Maryland, 20910) was reagent grade as it was only used as a rinsing solvent. Throughout the analysis, all glassware, blenders, etc. were washed after each use with hot, soapy water, dried, and rinsed three times with acetone and/or petroleum ether to remove contaminants. All vessels were covered with aluminum foil until used.

As mentioned, a 20g (approximate) aliquot was chosen for analysis

as suggested by Krantz, et al, 1970. The remainder of the contents were frozen for future use. The aliquots were mixed in a blender with a 3:1 mixture of a dessicant agent to egg contents, until a free flowing powder was obtained. The dessicant was a 9:1 mixture of anhydrous sodium sulfate and QUSO, G-32 (microfine precipitated silica) which keeps the mixture from lumping (Gulf Breeze Laboratory, 1968). It is available from Philadelphia Quartz Co., Public Ledger Building, Philadelphia, Pennsylvania. This mixture plus contents were wrapped in aluminum foil and frozen until just prior to analysis.

Samples were removed from the freezer and placed in pre-extracted Whatman cullulose extraction thimbles (43mm x 123mm) which are available from Scientific Products, 100 Raritan Center Parkway, Edison, New Jersey, 08817. Each thimble was filled with a plug of glass wool to prevent spattering of contents.

All thimbles and glass wool were extracted before use for seven hours in a Soxhlet apparatus with petroleum ether. Samples were placed in the Soxhlet apparatus and extracted with petroleum ether for seven hours. During extraction, lipids and pesticide residues are concentrated in distillation flasks.

After extraction, distillation flasks were fitted onto a Roto-vac (Fisher Scientific) and partially submerged in warm water. Hexane was evaporated and the pre-weighed distillation flasks were reweighed to determine weight of extracted lipids.

Lipids were transferred with 3x10ml washings hexane saturated with acetonitrile into a 250ml separatory funnel with a teflon stopcock. Distillation flasks were then rinsed with 50ml of acetonitrile saturated with hexane, and this solution was added to the separatory funnel.

Shaking the separatory flasks produced an emulsion between the hexane and acetonitrile. After sitting for several minutes, the solvents partitioned, with hexane containing egg lipids on top and acetonitrile containing the pesticides on the bottom. Difficult emulsions were broken by using a heat gun for several minutes. Acetonitrile was drawn off into a 500ml Phillips beaker. Hexane was repartitioned with 25ml of acetonitrile which was also drawn into the beaker. Beakers were placed on a slide warmer overnight to allow for evaporation of acetonitrile.

Chromatography columns (10mm x 400mm) were prepared with 120mm of Florisil (60-100 mesh) which was obtained from the Floridin Company, Berkeley Springs, West Virginia. Florisil was checked for pesticide retention with pesticide standards. Addition of 3.5% water partially deactivated the Florisil so that no pesticides were withheld by the column. Florisil columns were topped with 5cm of anhydrous sodium sulfate to remove any water and to hold the Florisil in place. Columns were packed by topping the glass column. Each column was checked for channels in Florisil by adding 25ml of petroleum ether.

Residues from acetonitrile were transferred to the Florisil column by 3x10ml hexane rinsings. Florisil binds lipids, egg pigments and pesticides. Pesticides are eluted into 500ml distillation flasks from the column by addition of 200ml of a 15% solution of ethyl ether in hexane or petroleum ether. Flasks are filled on the Roto-vac and contents evaporated to 1-2ml. This, along with several washings, was transferred to 10ml conical centrifuge tubes. Tubes were placed on a slide warmer overnight to allow for evaporation of hexane and ether. Thin layer chromatographic separation of residues followed the method of Mulhern (1968). Thin layer chromatography plates were prepared using a Desaga apparatus (available

from Brinkman Instruments, Inc., Cantiagul Road, Westbury, New York). A slurry was prepared by mixing 30g of silica gel with 55ml of water. This was then applied as a 250 micron layer to the plates. A special refined grade of silica gel from Brinkman (Macherey, Nagel & Co., Silica Gel G-HR) with a calcium sulfate binder was used. Plates were air dried and activated at 120^{o}C for 30 minutes before use.

Residues were dissolved in 100ul of 15% ethyl ether and hexane. Residues were spotted along the origin line of plate using a micro syringe. Centrifuge tubes and syringe were rinsed with another 100ul of the ether mixture. A standard pesticide solution containing o'p' DDE, p'p' DDE, o'p' DDD, p'p' DDD, o'p' DDT, p'p' DDT, dieldrin, endrin, lindane, heptachlor epoxide, Dichlorbenzophenone, mirex and Aroclor 1254 (Monsanto) were spotted at one end of the plate so that it would not come in contact with the egg residues. These pesticides were chosen because they have often been found in eggs in other similar studies. Care was taken to allow sufficient margins on either side of plate to permit even development in chromatography tank.

The plate was placed in a tank with 200ml of developing solution (195ml hexane + 4ml ethyl ether) and was allowed to develop for 15cm from the origin line. The portion of the plate containing the sample was carefully covered with aluminum foil. Standards were sprayed with a silver nitrate solution containing 10g of silver nitrate, 500ml of ethanol, 500ml of acetone and 10 drops of 30% hydrogen peroxide. Standards were then exposed to a short wave ultra violet light until brown.

Location of the standards facilitated division of the plate into four horizontal fractions. Fraction one, the bottom zone, contained dieldrin, endrin, heptachlor epoxide and 4,4 dichlorobenzophenone (DCBP). Fraction

two contained o'p' DDD, p'p' DDE and some dieldrin. Fraction three contained o'p' DDT, p'p' DDT and o'p' DDE along with some peaks of Aroclor 1254.

Each horizontal zone of silica gel and pesticide was collected from the thin layer plate by means of a Chromoflex Sample Recovery Tube (K.H600) containing a fritted disk from Brinkman. Pesticides were eluted from the recovery tubes by 3x20ml washings of hot benzene, the result being four tubes of pesticides dissolved in benzene for each egg sampled.

The final step of analysis involved the use of the gas chromatograph for quantitative and qualitative measurements of the residues. The instrument used was a Hewlett-Packard 5750 Research Gas Chromatograph, equipped with an electron capture unit with a Tritium detector. Electron capture is especially designed for more polar molecules, particularly those with halogens. Operating conditions may be found in Appendix A. Chromatographic information was printed and a strip chart with a disk integrator for measuring peak area.

Two gas liquid chromatography columns were used. Most of the identification and quantification was done with a 4 ft. x 3mm I.D. glass column packed with 3% QF-1 (mesh size 80/100). This column gave good and moderately fast separation of pesticides with the listed parameters (Appendix A). A second column, 6 ft. x 3mm glass column packed with 3% OV-1 chromasorb WHP (mesh size 100/200) was used for confirmation of pesticides identified by QF-1 Nine eggs were confirmed with the OV-1 column. Both columns were obtained from Hewlett-Packard (6707 Whitestone Road, Baltimore, Maryland). Injections were unusually concentrated but a high sensitivity permitted measurement of most pesticides down to the level of 0.01 ppm.

A standard pesticide solution was prepared with a concentration of

1 ug per ul for most pesticides but those pesticides which gave low reference peaks were concentrated to 2 ug per ul. All pesticides were obtained from Supelco, Inc., Bellefonte, Pennsylvania, 16823, with the exception of Aroclor 1254 (Monsanto) which was obtained through the courtesy of W. L. Reichel, Patuxent Wildlife Research Center, Laurel, Maryland. Reference of pesticide as to chemical name, source, lot number and ng per ul in standard solution may be found in Appendix B. A separate solution of Aroclor 1254 was prepared (10 ng per ul) for a standard.

The gas chromatograph was put in operating condition and left running for 24 to 48 hours to allow for stabilization before using. Standards were injected at least twice before any samples were injected in order to insure equilibration. After the injection of 20 samples (5 eggs), standards were injected in order to account for any changes in chromatographic parameters.

Gas chromatographic profiles were qualitatively analyzed by measuring retention time of the peaks and comparing these values with standard profiles. Relative retention times to dieldrin (1.00) may be found in Appendix C. Each pesticide has one peak with the exception of Aroclor which has 15 peaks as it is a mixture of chlorinated terphenyls. Five of the more prominent Aroclor peaks: #3, #6, #10, #11, #13, were used for positive identification of Aroclor.

Pesticides were quantitized by measuring peak heights from the preceding base line and comparing these values to standard peak heights. Quantification of Aroclor was made by obtaining the sum of the heights of the five previously mentioned peaks and comparing this value to the

sum of the same peaks in the standard. Peak heights were measured with a Mintutoyo Dial Micrometer to the nearest 0.01mm.

Quantitation of pesticides in ppm was achieved by the use of the following formula:

$$PPM = \frac{(\text{ml. sample})\ (\text{ng pesticide in standard})\ (\text{peak height of sample})}{(\text{wt. of sample in grams})\ (\text{sample})\ (\text{peak height of standard})}$$

Where:

ml = volume of sample at time of injection

ng = pesticide in standard usually i x ng pesticide per

peak height of sample - height of peak on recording paper (mm)

weight of sample in grams = weight of aliquot (g) fresh weight basis

sample = amount of sample injected (i) into chromatograph

peak height of standard = height of standard peak on recording paper (mm)

One reason for use of thin layer chromatography zonal separation was because peak #5 of Aroclor 1254 has the same retention time as p'p' DDE. Fortunately, this is not one of the more prominent peaks of Aroclor 1254, but this peak was found in fractions 1 and 2. To test the degree of contamination in section 2, six plates were streaked with Aroclor 1254 and developed as mentioned above. Fractions 1 and 2 were analyzed in the gas chromatograph.

Most (60%) of peak #5 of Aroclor 1254 is present in the first section but some also contaminates the second section (40%), where p'p' DDE is found. Other studies have shown that there is little interference of 1254 with DDE unless the ratio is 15:1 (Aroclor 1254:DDE) when using peak height for quantitation (Riechel, et al., 1969).

Four blanks were run through the entire procedure to test for contaminating peaks. Several peaks were found in close association with the

solvent peak. These peaks are most likely from the sodium sulfate (Levi and Nowicki, 1972) and do not interfere with identification.

To determine the recovery efficiency of the analytical procedure, 10 chicken eggs were injected with a known concentration of pesticide mixture. These eggs plus 5 control eggs were analyzed using the above procedure. Control eggs showed traces of DDE and dieldrin. However, analysis of the injected eggs did not show any pesticides other than those found in the control eggs. It was later discovered that equal mixing of the injected pesticides had not occurred and most of the pesticide was probably in the shell membrane. Due to unavailability of solvents, controls and experimental eggs were not repeated. Riechel, et al. (1969) found recovery with this analysis procedure to be 95% for DDE, 102% for DDD, 110% for DDT and 106% for dieldrin, 112% for heptachlor epoxide and 75% for DCBP for eagle tissue fortified with pesticides. Another study using Carbon-14 labeled p'p' DDT and dieldrin, found 80% recovery for DDT and 79% for dieldrin (Prouty and Cromartie, 1970).

Presentation and Analysis of Data

Data are presented in the form of Robert Kennedy (1972) and Gary Seek (1974) in which Tidewater Virginia and the Chesapeake Bay are divided in smaller study areas. In the system: J.R. = James River; Y.R. = York River; M.B. = Mobjack Bay; N.P.C. = New Point Comfort; R.R. = Rappahannock River; F.B. = Fleet's Bay; P.R. = Potomac River; Ocean = Ocean side of the Eastern Shore; Bay = Bay side of the Eastern Shore.

Eggshell parameters (Table 1) for each study area are expressed in the form of mean $\pm$ standard error. The percentage of change from the pre 1947 shell parameters is found below each mean in order to conform with existing data (Anderson and Hickey, 1972a). Student's test for non-paired

data was used to compare differences in thickness of entire eggs and fragments for each study area. A Wilcoxon two sample test was used to compare thicknesses of eggs with known developmental stages to eggshells from hatched chicks and from broken eggs. Student's test for paired data were used to relate the means of first and second clutch eggshell parameters.

Data for pesticide residues are expressed on a ppm wet weight basis (Appendix D) rather than a lipid basis (Stickel, et al., 1973). Development of the egg alters lipid levels which could bias the data. The weight of each aliquot and the weight of the lipid found in that aliquot are also displayed in Appendix D.

Various attempts were made to relate changes in eggshell thickness to pesticide residues. Linear correlation is inappropriate since eggshell thickness values were not normally distributed on normal probability paper. For this reason, Spearman's rank test (Siegel, 1956) was used to relate shell thickness and the levels of p'p' DDE, dieldrin, and Aroclor 1254. Linear regressions were performed in order to relate shell thickness and the levels of the above pesticides plus the log dieldrin and log p'p' DDE levels. Predictive values for regressions were poor so these data are not included.

A Wilcoxon two sample test was used to compare DDE levels in the manipulated eggs with and without development. This test was also used to relate levels of p'p' DDE with successful nests (nests which hatch at least one young) and unsuccessful nests. Failure of this test resulted in the use of the less powerful median test for association of these data. Homogeneity of data was tested for pesticide levels and shell parameters by using a Kruskal-Wallis test (Siegel, 1956).

RESULTS

Data for the eggshell parameters of shell weight and thickness index are found in Table 1. Shell weight for the entire study area averaged 5.91 g. within a range of 4.21 - 7.51 g. Shell weight values for all the study areas were not significantly different when tested with a Kruskal Wallis test (Siegel, 1956). Thickness index measurements are one way of overcoming the variability of the shell thickness and provide a means of comparison with museum specimens (Ratcliffe, 1967). Since thickness index values are a function of the eggshell weight, these values showed trends similar to eggshell weight values. The mean shell thickness index was 2.16 with range from 1.61 to 2.59.

The mean values for shell thickness for the entire study area was 0.420mm. Eggshell thickness measurements ranged from a low value of 0.299mm to a high of 0.522mm. Values for shell thickness in each study area include measurements of shells from entire eggs and eggshell fragments. Use of a t-test for non-paired data showed no difference between the shells and fragments for each study area. Data were tested for homogeneity between study areas with a Kruskal Wallis test and were found to be heterogeneous. Further use of a Wilcoxon Two sample test showed that there was a significant difference between the areas with the most shell thinning (York River and Mobjack Bay) when compared to the rest of the study areas.

As might be expected, the mean eggshell thickness for fragments and broken eggs (μ = .403mm) was significantly ($p<0.05$) thinner than

those eggs showing development (u = .423) when compared with a Wilcoxon Two sample test (Stelle and Torrie, 1960). However, the mean eggshell thickness was not significantly different from the mean thickness of those eggs showing development or from the mean thickness of fragments and broken eggs, using the same test.

Table 2 includes measurements of length (mm), breadth (mm) and volume (ml) of all entire eggs collected. Pre-1947 measurement comparisons for these parameters are expressed in the form of mean $\pm$95% confidence limits (data courtesy of J. J. Hickey). Data for the current study areas are, however, expressed in the form of mean $\pm$ standard error. These data were not found to be significantly different for the study areas when small samples (3 or less eggs) were eliminated. Current data are not readily comparable to the pre-1947 data (standard deviation and all values for the pre-1947 data were unavailable). However, comparison of the mean of each eggshell parameter with pre-1947 means does not reveal any striking differences.

Non-significant differences were also found for two other aspects of eggshell thickness. First of all, comparison of eggshell thickness from first and second clutches of eggs (Appendix E) using a t-test for non-paired data did not reveal any significant differences in eggshell thickness between first and second clutches. First clutch eggs were those brought into the laboratory (manipulated eggs) for incubation, while second clutch eggs represent the renesting attempts of the females whose first clutches were removed. Secondly, measurement of eggshells without including shell membranes did not reveal any significant relationships which have not been previously mentioned. It had been hoped that this procedure would perhaps eliminate some of the

TABLE 1

COMPARISON OF OSPREY EGGSHELL DATA:

EASTERN UNITED STATES PRIOR TO 1947 (ANDERSON AND HICKEY, 1970a) VS. VIRGINIA. 1972

	Number, Means + S.E., % Change					
Area	Shell Weight (g) N		Shell Thickness (mm)* N		THICKNESS INDEX N	
East U.S.** Pre 1947	365	7.07 ± 0.04	963	0.505 ± 0.004	365	2.57 ± 0.01
J.R.			1	0.388 ± 0.000 -23.2%		
Y.R.	11	5.49 ± 0.26 -22.4%	14	0.392 ± 0.011 - 22.4%	11	2.01 ± 0.07 -21.8%
M.B.	7	5.79 ± 0.19 -18.1%	13	0.390 ± 0.010 -22.8 %	7	2.04 ± 0.04 -20.6%
N.P.C.	36	5.73 ± 0.10 -18.9%	58	0.432 ± 0.005 -14.5%	36	2.16 ± 0.04 -16.0%
R.R.	14	6.23 ± 0.17 -11.0%	18	0.421 ± 0.009 -16.6%	14	2.29 ± 0.06 -10.9%
F.B.	19	6.20 ± 0.20 -12.3%	26	0.428 ± 0.009 -15.2%	18	2.20 ± 0.05 -14.
P.R.	3	5.56 ± 0.44 -21.4%	10	0.417 ± 0.009 -17.4%	3	2.10 ± 0.09 -18.3%
Ocean	S	6.27 ± 0.15 -11.3%	20	0.417 ± 0.008 -17.4%	8	2.17 ± 0.05 -15.6%
Bay	1	6.60 ± 0.00 -6.6%	3	0.447 ± 0.023 -11.5%		2.23 ± 0.00 -11.3%
Total	99	5.91 ± 0.07 -16.4%	163	0.420 ± 0.003 -16.8%	98	2.16 ± 0.02 -15.9%

* Data includes measurements of entire eggshells and fragments
** Data are in form of mean ± 95% C.L.'s

variability in thickness measurements which is due to changes in thickness of the shell membrane.

Nature of Contents

Table 3 gives a classification of pre-stages of embryonic development for the manipulated eggs. Presumably most of the first clutch manipulated eggs should have hatched under laboratory incubation. However, a relatively low percentage (19.1%) of the eggs hatched. Most of the embryonic mortality (39.7%) was confined to the third and fourth week of development. Eggs showing no development may be unfertilized eggs or eggs with embryos which died early in development. Eggs from the second clutches which were left in the field to be incubated by the female show a higher percentage of non-development, but also a higher hatching success (26.4%) than those eggs from first clutches. Therefore, the somewhat lower hatching success of the manipulated eggs from the first clutches may have been due to improper incubation.

There were no correlations between stages of embryonic development in weeks and the levels of pp'DDE, pp'DDT, pp'DDD, dieldrin or PCB's. However, there was a significantly (p 0.05) greater amount of PP'DDE in those eggs which had no development when compared with a Wilcoxon Two sample test to eggs which show some degree of development. (Refer to Table 3 and Appendix D).

Table 4 presents the developmental stages of the 58 non-manipulated eggs collected from the field. Most of these eggs were collected after 35 days or longer of incubation as it was assumed that they would not hatch. Fifty-one per-cent of the eggs did not show any obvious development. This increased percentage of the undeveloped eggs could have

TABLE 2

COMPARISON OF EGG MEASUREMENT DATA *

AREA	N	LENGTH (cm)	BREADTH (cm)	VOLUME (ml)
EAST U.S.** PRE 1947	963	6.05 ± 0.02	4.59 ± 0.07	68.7 ± 12.17
J.R.	1	6.24 ± 0.00	4.75 ± 0.00	71.7 ± 0.00
Y.R.	13	6.09 ± 0.70	4.50 ± 0.57	61.5 ± 1.80
M.B.	7	6.15 ± 0.57	4.63 ± 0.80	63.7 ± 3.47
N.P.C.	42	5.89 ± 0.45	4.50 ± 0.26	58.1 ± 1.14
R.R.	16	6.03 ± 0.66	4.55 ± 0.37	62.0 ± 1.16
F.B.	19	6.11 ± 0.73	4.60 ± 0.53	64.8 ± 1.89
P.R.	3	5.98 ± 1.03	4.41 ± 0.79	60.7 ± 3.47
OCEAN	8	6.23 ± 0.85	4.65 ± 0.73	66.5 ± 1.13
BAY	3	6.12 ± 0.56	4.58 ± 0.60	64.4 ± 1.78
TOTAL	112	5.97 ± 0.56	4.54 ± 0.72	61.4 ± 1.67

* DATA ARE EXPRESSED AS MEAN ± S.E.
** DATA COURTESY OF J. J. HICKEY ARE EXPRESSED AS MEAN ± 95% CONFIDENCE LIMITS.

TABLE 3

NATURE OF EGG CONTENTS OF MANIPULATED NESTS

SAMPLE SITE	FIRST CLUTCH								SECOND CLUTCH								
	AGE OF DEVELOPMENT (WEEKS)								AGE OF DEVELOPMENT (WEEKS)								
	N	NONE	1	2	3	4	5	HATCH	N	NONE	1	2	3	4	5	HATCH	LOST
CB-6	3	1		2					2				1				1
CB-13	3					2			0								
FB-14	3				2	1			3							2	1
FB-24	3				1	1		1	2	2							
FB-28	3			1	1	1			2	1						1	
FB-34	3	1	1	1					0								
M-31	3					1	2		3					1		2	
NPC-32	3				1		1	1	2							2	
M-4	3	1	1					1	3							2	1
M-7	4	1	1	1				1	2								2
M-10	4	1	1	1		1			3	2							1

TABLE 3 (Cont.)

SAMPLE SITE	FIRST CLUTCH								SECOND CLUTCH								
	AGE OF DEVELOPMENT (WEEKS)								AGE OF DEVELOPMENT (WEEKS)								
	N	NONE	1	2	3	4	5	HATCH	N	NONE	1	2	3	4	5	HATCH	LOST
M-11	3	1				2			2							1	1
M-24	3					1		2	2							2	
NPC-16	3					1		2	3							3	
NPC-17	3					1		2	3							2	2
NPC-23	3	3							3	1		1					1
NPC-35	4	1	1			1			3	2							
NPC-40	3	1			2				0								
NPC-43	3				3				0								
WN-1	3				2				2								1
TOTAL	63	11	5	6	12	13	4	12	38	8	0	1	1	1	0	17	10
PERCENT		17.5	7.9	9.5	19.1	20.6	6.3	19.1		21.0	0	2.6	2.6	2.6	0	44.7	26.4

TABLE 4

STAGE OF EMBRYONIC DEVELOPMENT OF OSPREY EGGS COLLECTED - 1972

NON-MANIPULATED NESTS

AREA	NO. OF EGGS WITH NO DEVELOPMENT APPARENT	STAGES OF DEVELOPMENT (WEEKS) 1	2	3	4	5
J.R.	1(100)*					
Y.R.	4(67)		2(33)			
N.P.C.	9(64)		1(7)	2(14)	2(14)	
R.R.	6(46)		2(15)	3(23)	1(8)	1(8)
F.B.	4(46)	3(33)	1(11)		1(11)	
P.R.	1(33)	1(33)		1(33)		
OCEAN	4(46)	2(22)		1(11)	1(11)	1(11)
BAY	1(33)	1(33)	1(33)			
TOTAL	30(51)	7(12)	6(10)	8(14)	5(9)	2(3)

* NUMBERS IN PARENTHESES INDICATE PERCENT

resulted from a larger number of infertile eggs in the sample, but most likely reflects a sampling bias. With the exception of the fifth week of development, embryonic death occurred in a uniform pattern throughout the developmental period. No relationships were established between stages of development and levels of pesticides previously mentioned. Unlike the manipulated eggs, there were no differences in residues of pp'DDE between eggs with no development and eggs with some degree of development in the non-manipulated eggs.

In order to compare further the unhatched non-manipulated eggs from the field, nests from which these eggs came were categorized. Nests which hatched at least one young were termed successful, while nests which hatched no young were called unsuccessful. Comparisons of eggs from these nests with a median test showed a significantly ($p<0.01$) higher level of pp'DDE in the eggs from the unsuccessful nests.

Pesticide Residues

Residue profiles from the 85 osprey eggs analyzed are found in Appendix D. Each study area has been considered separately and a mean for each residue within the study area is presented. Data for chlorinated hydrocarbon residues are expressed on a ppm net weight basis rather than on a lipid basis (Stickel, et al., 1973). Adjusted net weight of the aliquot (see methods section) and actual lipid weight for that aliquot are also displayed in Appendix D.

Residues of pp'DDE were found consistently throughout the entire study area. The op' isomer of DDE was not found in this study. Possible residues of this isomer were masked by a persistent peak of Aroclor

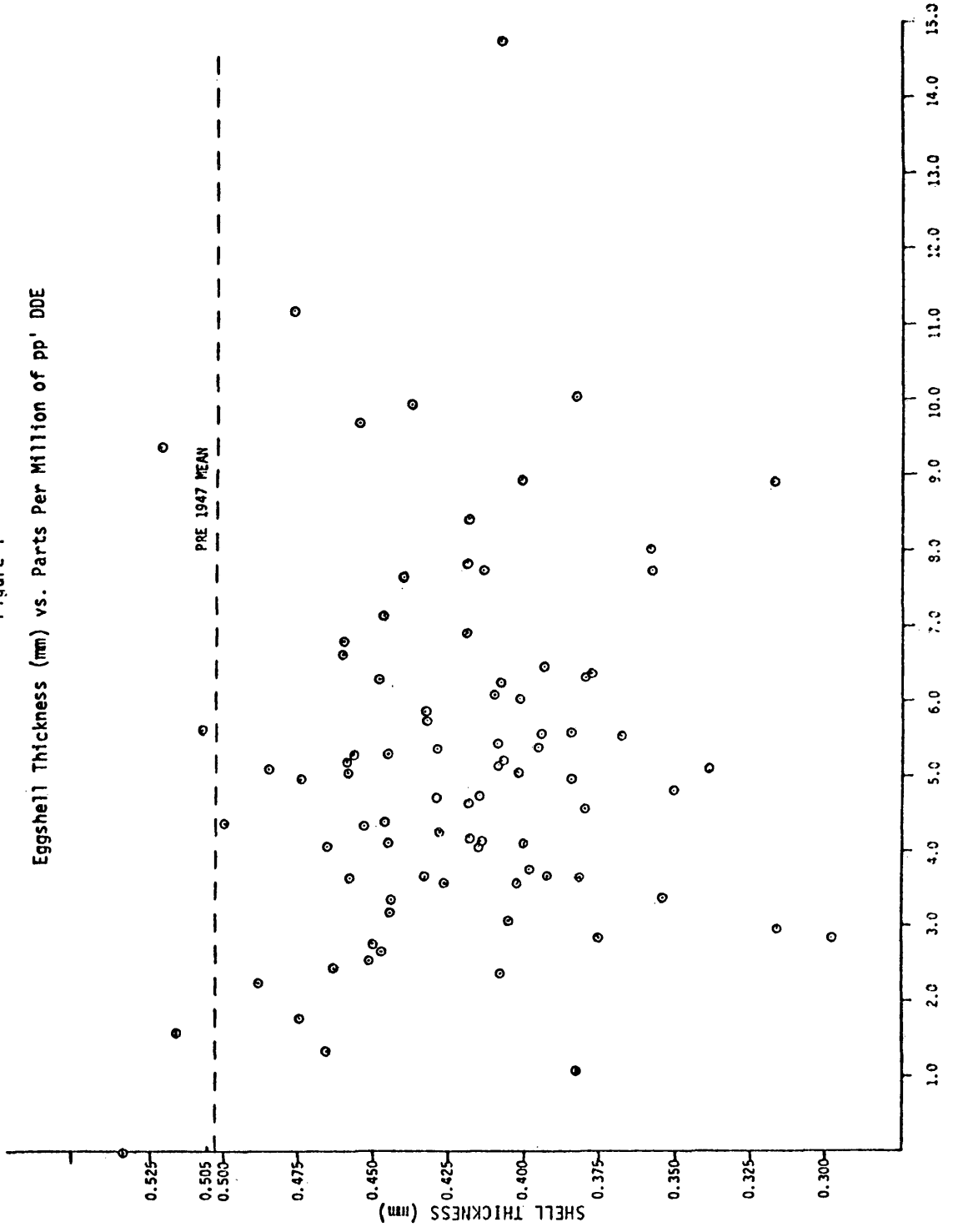
1254. Therefore, characterization of op'DDE pesticide was not certain. Residue levels of pp'DDE ranged from 0.83 to 33.28 ppm. Contamination by Aroclor 1254 may perhaps bias levels of pp'DDE since 40% (peak height) of the Aroclor 1254 peak #5 was found in the same thin layer fraction as pp'DDE. Since the Aroclor 1254 peak #5 is relatively small, interference with DDE levels is not thought to be severe.

No relationships were established between residues of DDE and any of the shell parameters. Linear correlation is inappropriate since residue levels of pp'DDE are not normally distributed on normal probability paper. Data were tested using a Spearman Rank Correlation Test (Siegel, 1965). Comparisons were made between either the DDE residues or log DDE residues and shell thickness or thickness index. Linear regressions showed little predictive values since r values were very low and y intercepts (shell thickness) were not similar to pre-1947 values. Figure 1 is a graphical presentation of the relationship between eggshell thickness and pp'DDE residues.

Two other residues of the DDT complex were also found. Most of the eggs contained pp'DDD. Residue levels in eggs ranged from 1.00 to 21.54 ppm. No relationship was established between shell parameter changes and residues of pp'DDD. In general, residues of pp'DDT were found to be low with respect to residues of pp'DDE and pp'DDD. Approximately 30% of the eggs analyzed showed only traces of pp'DDT. Residue levels ranged between trace quantities (below .05ppm for DDT) and 9.42 ppm residues of pp'DDT were not found to be related to shell thickness changes when analyzed with a Spearman

Figure 1

Eggshell Thickness (mm) vs. Parts Per Million of pp' DDE



Rank Correlation test. Due to the possible masking of Aroclor 1254, residues of neither op'DDD or op'DDT were detected.

Residues of Aroclor 1254 were the highest of any of the single chlorinated hydrocarbonssampled. The levels ranged from no residues to 39.23 ppm. In most cases, residues of Aroclor 1254 were comparable to the total of all DDT metabolites found in the egg. Only residues of Aroclor 1254 were found, but many other Aroclors (Aroclor 1232, 1242, 1248, 1260, 1262) are used in industry. No attempt was made to identify any of these other compounds.

Residues of Aroclor 1254 are difficult to identify and quantify because of their chemical nature. Since Aroclor 1254 is a mixture of chlorinated biphenyls, fifteen peaks result on the gas chromatograph profile. Many of these peaks overlap peaks of several chlorinated pesticides and in some cases elimination of overlap was not aided by the zonal separation of thin layer chromatography. Residues of Aroclor 1254 were not related to shell thinning or any shell changes when compared with a Spearman Rank Correlation test.

Dieldrin was another consistent contaminant of the eggs analyzed. Levels ranged from 0-3.86 ppm. Dieldrin was found to be present in both thin layer fractions #1 and #2. Levels of Dieldrin and log of Dieldrin residues were not related to shell thinning or thickness index when compared with a Spearman Rank Correlation test.

Heptachlor epoxide which is a metabolite of heptachlor and DCBP (dichlorobenzophenone) which is a metabolite of DDE, were usually found only as trace quantities ($<$0.05ppm). Since levels of these residues were low, determination of any relationships with egg shell thinning and development were not possible.

Two other pesticides were also infrequently found. Lindane was found in 11 cases and ranged between 0.03 and 0.16 ppm. Endrin was also found in 3 cases and ranged between 0.07 and .90 ppm.

Residues of mirex, aldrin, heptachlor, methoxychlor and kelthane were not found in any of the eggs analyzed. However, this is not to say these pesticides were not present since pesticides often have similar retention times on some gas chromatograph columns. Only two columns were used in this study and several more should be used for confirmation of these uncommon residues.

DISCUSSION

The mean value for shell thickness for the entire study area represents a 16.8% decrease in shell thickness from the pre-1947 values (Anderson and Hickey, 1970a). This mean reduction in shell thickness is comparable to the 16.4% shell thinning found by Weimeyer for ospreys in Maryland and Virginia in 1972 (Weimeyer, 1972 unpublished data). When comparing thickness measurement averages to other studies, only the average thicknesses from the study areas of the James and York Rivers and Mobjack Bay approach the 22% shell thinning which Spitzer (1972) found in the ailing Connecticut and New Jersey populations. These same study areas are also comparable to the 21% shell thinning which Anderson and Hickey (1970a) found in osprey eggs from the Northeast. The decrease in eggshell thickness is also evident since eggshell fragments from broken eggs were significantly ($p<0.05$) thinner than eggshells from eggs with some degree of development. Similar results were found in the eggs of the North American Cooper's hawk, Accipiter cooperi (Snyder, et al, 1973).

The poor hatching success of the manipulated eggs may be due to several factors. The most probable reason for hatching failure would be the improper humidity levels in the incubators which were used (Seek, 1974). However, many studies have observed embryonic mortality in eggs from pesticide-dosed adults. For example, Heath et al. (1969) found that hatching success in mallard ducks was reduced up to 30% after two years of exposure of female to 40 ppm DDE in the diet. Most of the

embryonic mortality in the present study was confined to the final stages of incubation. Other studies have shown that mortality due to pesticides occurs either very early in development due to absorption of yolk sac material or mobilization of fat reserves (Koeman et al., 1967; Cooke, 1971, reviewed in Cooke, 1973) or during the later stages of incubation due to increased rate of lipid uptake (Guthrie and Donalson, 1970).

Pesticide residue levels in avian eggs are subject to several sources of degradation so that obtained residue profiles do not necessarily present an accurate evaluation of the pesticides present in the eggs. For example, examination of the eggs sampled shows a considerable percentage of embryonation in the eggs analyzed. Levels of DDT may be reduced by as much as 50% in eggs with development (Patuxent Wildlife Research Center, personal comm.). However, the DDT is not lost, but transformed to either the DDE or DDD metabolites. Since many of the eggs analyzed were addled, putrefaction of eggs may also be an important factor in degrading pesticides. However, one study found no statistical differences in the recovery of DDE, DDT, or DDD from putrefied eggs when compared to controlled eggs (Mulhern and Reichel, 1970). This study also found no conspicuous alterations in the levels of pesticides as a result of the putrefaction process. Individual variation between eggs is to be expected since residue levels in avian eggs are only indications of the magnitude of pesticides in the body of the female bird which are a result of such factors as feeding patterns, pesticide pollution at nest site and individual metabolism (Cecile et al., 1972).

Despite all of the above factors which may be responsible for the large variability of pesticide residues of eggs, the mean residue levels in avian eggs provide the only available indication of the relative

concentrations of pesticides in osprey populations. For example, average levels of DDE from the York River, Atlantic Ocean side of the Eastern Shore, Mobjack Bay and New Point Comfort study areas (Appendix D) are comparable with the residue levels from Connecticut reported by Stickel et al., (1965) who found a 6.5 ppm avg. total DDT residues and Ames (1966) who reported 5.1 ug/ul of DDE. Weimeyer also found an average of 5.1 ppm of DDE in 3 Potomac River eggs (1972) Weimeyer, unpublished information).

Residues of DDE are the most common residues found in avian tissues (Hickey and Anderson, 1968), since they are the matabolic products of DDT and may comprise 40% of analyzed residues when adult birds have been fed a daily diet of DDT (Cecile et al., 1973). DDE is more stable to degradation than DDT and DDD in avian tissues (Abou-donia and Mengel, 1968). Since it also has a long half life in pigeons of 250 days (Bailey et al., 1969) it is not difficult to see why levels of DDE are often high in relation to other pesticides.

As mentioned earlier, DDE may be responsible for shell thinning as indicated in many studies. Many other scientists have found various relationships between residues of DDE and changes in shell thickness or thickness index (Cade et al., 1971; Fyfe et al., 1969; Enderson and Berger, 1970; Anderson and Hickey, 1970b; Risebrough et al., 1970; Blus et al., 1971, 1972; Spitzer 1972; Snyder et al., 1973).

In this study, there were no relationships established between levels of DDE and changes in any of the eggshell parameters. It is noteworthy that DDE residues of similar magnitude to those in this study have been related to shell thinning of osprey eggs in several studies (Spitzer 1970; Weimeyer 1972 unpublished data). Under ideal

sampling conditions residue levels may be difficult to link with eggshell thinning because contrary to popular belief, increased concentration of residues does not necessarily result in a linear decrease in shell thickness. Anderson et al. (1969) found that with pp' DDE there was no minimum effective level concerning eggshell changes. For example, another study found similar results since the calculated percentage of thinning in brown pelicans was 4.2% at 1 ppm DDE and only 0.4% at 100 ppm DDE (Blus et al., 1972). Residues of DDE were found to be related to embryonic mortality since eggs from unsuccessful non-manipulated osprey nests contained significantly ($p<0.05$) higher levels of DDE than did those eggs from successful nests when a median test was used. Similar results have also been observed with North American Cooper's hawk eggs (Snyder et al., 1973).

Residues of pp' DDD were also consistently found in the eggs analyzed. The relatively high levels of DDD are the result of metabolization of DDT and DDE. DDD is less toxic to several species of experimental birds when compared to DDE and DDT (Stickel and Stickel, 1969; Stickel, 1969) and has a shorter half life in pigeons of (24 days) when compared to DDE (Bailey et al, 1969).

Reproductive success of mallard ducks was impaired with DDD, but impairment was not as severe as that caused by DDE (Heath et al., 1969). The same study did not find any shell thickness changes as a result of increased levels of DDD. No relationships between any shell parameter measurements and levels of DDD were found in this study. Residues of the op' isomer of DDD were not detected in this study. This may be due to interference of other pesticides or Aroclor (Lamont et al., 1970).

Mean levels of DDT for most of the study areas were comparable to

the 0.70 ppm Weimeyer found for the Potomac River in 1972 (Weimeyer 1972, unpublished data). Levels of DDT are lower than those of DDE and DDD for perhaps several reasons. First, metabolism of DDT residues in the body of the female bird would be a major cause of low DDT levels transmitted to the egg (Cecile et al., 1973). Second, DDT may be metabolized due to the development (Patuxent Wildlife Research Center, personal comm.; Lamont et al., 1970). Third, because of limited use of DDT in the past few years, there is less chance of contact between the birds and the pesticide. Finally, the half life of DDT in birds (pigeons) is short (28 days) when compared to DDE (Bailey et al., 1969). DDT has been postulated to cause shell thinning in several studies. Bitman et al. (1969) found that feeding either op' or pp' DDT in the diet caused eggshell thinning with the first eggs laid by Japanese Quail. However, in most studies, evidence tends to rule out any direct effect of DDT on shell thinning. Cecile et al. (1972) found no shell thinning in white leghorns by feeding pp' DDT in several concentrations. Studies which have reported shell thinning from DDT have used technical grade DDT which may contain impurities which might be the reason for the resultant thinning (Cooke, 1973). The probable cause of DDT induced shell thinning is the level of DDE which exists as a metabolic of DDT since residues of DDE may comprise 40% of the residues analyzed in eggs from birds which have been fed solely DDT (Cecile et al., 1973). Like the above findings, no relationship between residues of DDT and shell thinning or embryonic mortality could be detected in this study.

Residues of Aroclor 1254 were higher in this study than those levels found by Weimeyer for Maryland in 1972 (Weimeyer 1972, unpublished data).

Aroclor is found often in nature since it is a highly persistent chlorinated compound that is widely used in industry (Risebrough et al., 1968; Pichirallo, 1971). When compared to Aroclors with less chlorine, Aroclor 1254 is more stable and more resistant to metabolism in avian tissue (Bailey and Bunyan, 1972; Peakall and Lincer, 1970).

In this study, Aroclor 1254 was not related to changes of any of the shell parameters. Because Aroclor 1254 levels have been closely correlated with levels of DDE in several studies in nature, it is often difficult to determine whether Aroclor 1254 is related to shell thinning (Risebrough et al., 1970; Anderson et al., 1969). However, several feeding experiments involving Aroclor 1254 in the diets of experimental birds show that Aroclor has little effect on shell thinning (Peakall, 1971; Heath et al., 1970; Dahlgren and Linder, 1971).

Recently, Aroclor compounds have been suspected of reducing hatchability of eggs. Pheasants show decreased egg production and decreased hatchability when fed Aroclor 1254 (Dahlgren and Linder, 1971). Peakall also reports massive embryonic mortality in the second generation of doves fed Aroclor 1254 (Peakall, Lincer and Bloom, in press, reported in Spitzer, 1972). Since Aroclor 1254 may decrease hatchability of eggs and may increase embryonic mortality PCB's could play an important role in the reproductive failure of some raptorial species (Risebrough et al., 1968; Prestt et al., 1970; Spitzer 1972).

Another residue which is a persistant pollutant and was consistently found in this study is dieldrin. Mean residues of dieldrin from the study areas were generally much higher than 0.23 ppm average found by Weimeyer for 1972 (Weimeyer, unpublished data). The mean concentration of dieldrin is similar to the 0.66 ppm dieldrin level found in 10 eggs

from the Connecticut River (Spitzer, 1972). Residue levels of dieldrin for most study areas also approach the levels thought to be responsible for the decline of the golden eagle in Scotland (Lockie et al., 1969).

Despite the relatively high levels of dieldrin found in this study, no relationships were established between the concentration of dieldrin and changes in eggshell parameters. Other studies have also found that dieldrin has no effect on shell thinning. For example, feeding studies using ring doves, Streptopelia risoria, pheasants, Phasianus colchicus, and mallard, Anas platyrhynchos, did not find any shell thinning with dieldrin included in the diet (Peakall, 1970b; Dahlgren and Linder, 1970; Muller, 1971). However, the data are inconclusive since dieldrin may have some effect on shell thinning as shown in some other experiments since. Porter and Weimeyer (1969) found a mixture of dieldrin and DDT included in the diet of American kestrels induced shell thinning. Blus et al., (1971) also found a positive relationship with shell thinning and dieldrin, but DDE showed a more powerful and consistent relationship. Since dieldrin is more toxic than any of the Aroclors, it is possible that it may have different effects on different genera (Cooke, 1973).

Other residues which were commonly found in this study include heptachlor epoxide, DCBP, lindane and endrin. These residues are often found in analysis of avian tissues and eggs (Riechel et al., 1969; Keith and Hunt, 1966; Weimeyer et al., 1972; Greichus et al., 1973). However values in most cases would appear too low in concentration to be related to changes in shell parameters. In the case of lindane and endrin, residues were found too infrequently to be responsible for eggshell thinning.

Despite the many possible sources of variation which may alter relationships between pesticide residues and shell thickness, correlations between these two variables have been found in many studies even though the relationship was not proven in this study. Most often members of the DDT complex have been most responsible for the thinning, and since shell thinning has occurred on two continents simultaneously, it is most likely shell thinning was responding to the same environmental influence; namely, the widespread use of chlorinated pesticides.

Difficulty in relating shell thickness and chlorinated hydrocarbon residues in this study exists because of many sources of variability. Since this study is based on two variables, eggshell thickness and chlorinated hydrocarbon residues, factors which would alter either variable might also alter any existing relationships between them.

One important bias in this study was the non-random collection of eggs for analysis. Most of the non-manipulated eggs were collected long after their normal incubation time. Weaker eggs which presumably would have higher pesticicide residues were broken early in incubation and were lost from the sample (Anderson and Hickey, 1970a). This collection bias is also evident since eggshell fragments from broken eggs are significantly thinner ($p<0.05$) than eggshells from eggs with some degree of development. Those entire eggs collected for pesticide analysis would also by the same logic be expected to have lower residues and thicker eggshells. Presence of a large number of these eggs would bias thickness values above the mean thickness for all eggs in the study area.

Shell thickness changes may be due to several factors besides pesticide induced shell thinning. For example, one area which has been relatively ignored in the thinning question is the relationship of

development on eggshell thickness. Kreitzer (1973) suggests that calcium transfer from the eggshell to the developing embryo occurs in all avian species. The net effect of this transfer would be that eggs with greater development would have relatively thinner shells when compared to eggs with less development. Eggshell thickness may also be influenced by the position of the egg in the clutch laying sequence (Romanoff and Romanoff, 1949; Bitman et al., 1969).

Anderson and Hickey (1970a) do not believe their data on pre-1947 eggshells were affected by development. These data were used as a means of comparison with recently collected eggs in this study. Most of the eggs in the museum collection examined by Anderson and Hickey were collected early in development since the contents were removed through small holes. Many eggs in museum collections also denote the developmental condition. There would also be little effect on the position of the egg in the clutch laying sequence since museum collections have the entire clutches of eggs.

Another major source of variability in this study would result from the analysis procedure and technique. After three different cleanup operations and one thin layer chromatography separation it is highly possible that certain residue levels are distorted or lost. Hopefully, recovery of all pesticide residues is uniform.

There are also many variables which might affect the distribution of pesticides in the environment and the accumulation and removal of pesticides in the body of the female bird. Dispersal of pesticides in nature is heterogeneous. Residue levels may differ between habitat types and different areas of the same habitat (Keith, 1969). There is also a great deal of individual variability within each of the study

areas. This variability is best revealed by examination of the residue profiles from individual birds within the study area (Appendix D).

Although no differences in shell parameters were found between first and second clutches of the manipulated eggs in this study, the number of eggs was too small for any meaningful comparison. Bitman et al. (1969) did find that after a period of no laying in DDT dosed Japanese quail, eggshells were thicker in the second clutches when compared to the first clutches. In light of possible alteration on shell parameters, further study needs to be made concerning the effect of the double clutching management technique in ospreys (Kennedy 1971: Spitzer 1972; Seek 1974).

Further work is also needed to understand lipid and pesticide mobilization in the female birds during egg laying. There appears to be some ordination within the clutch for pesticide residue concentration. For example, examination of the clutches CB-13, WP-1, M-31, M-11, NPC-35, NCP-40, and FB-14 show, in most cases the same order of ranking for all pesticides residues. More work is needed to determine whether those eggs with the highest residues were laid first or last in the clutch-laying sequence. The clutch sequence was known for two eggs analyzed. The first egg of one clutch (CB-13-1) generally showed the lowest levels of residues in the clutch while the last egg laid in another clutch (M-10-4) tended to show the highest residues in the clutch.

If northern osprey populations are predictive of future trends, then declines in other populations along the Atlantic Coast might be expected. Since eggshell thinning values of the York River and Mobjack Bay are comparable to the degree of thinning found in the ailing Connecticut and New Jersey populations, complete reproductive failure might

be expected in these areas first. With the exception of the bay side of the Eastern shore, the York River and Mobjack Bay areas show the lowest productivity (fledglings per active nest for 1972 (Seek 1974).

Seek (1974) found the annual rate of decline in the lower Chesapeake Bay population to be 8.9% for 1972 and 5.3% for 1973. Continued annual declines of similar magnitude would eventually result in very low populations as discussed by Spitzer (1970, 1972) and Peterson (1969a, 1969b) or in an exterminated population as was the population around Avalon, New Jersey (Schmid, 1966).

Potential for recovery from shell thinning is found in certain California populations of the American kestrel (Anderson and Hickey, 1970a). There is also potential for recovery of reproductive success from pesticide poisoning for raptors. Lockie et al. (1969) found that between 1966 and 1969 reproductive success (number of young fledged) for the golden eagle was 69%. This represents a 38% increase from the reproductive success of the period from 1963 to 1965. Lockie also found a significant decrease in levels of dieldrin between 1963-1969.

Populations of long-lived birds such as the osprey in which reproductive failure is involved cannot be expected to respond immediately to changes in contamination levels in the environment. However, it is hoped that the recent 1972 ban on the use of DDT will eliminate many of the annually added residues in nature. In time decreasing levels of residues would eventually result in increased shell thickness and increased hatchability so that osprey populations along the east coast would recover to their former productive status.

APPENDIX A

OPERATING CONDITIONS FOR GAS CHROMATOGRAPH WITH A ^{3}H ELECTRON CAPTURE DETECTOR

Liquid Phase	3% QF-1	3% OV-1
Mesh Size	80/100	100/120
Carrier Gas (ml/min) 5% Methane in Argon	60	60
Purge Gas (ml/min) Nitrogen	90	90
Injection Port Temp. (°C)	230	230
Column Temp. (°C)	190	190
Attenuation	16	16
Electrometer Range	100	100
Pulse Interval usec.	50	50
Retention Time for Dieldrin (min)	7	7

APPENDIX B

REFERENCE DATA FOR PESTICIDES

PESTICIDE		SOURCE	LOT #	ng/ul of Standard
Common Name	Chemical Name			
p'p' DDD	(p'p')2,2-Bis(p-chlorophenyl-1,1-dichloroethane	Supelco	1575	2
o'p' DDD	2,2-Bis(o'p'-chlorophenyl-1,1-dichloroethane	"	04-9015	2
p'p' DDE	p'p'-dichlorodiphenyldichloroethane	"	1542	2
o'p' DDE	o'p'-dichlorodiphenyldichloroethane	"	-	2
p'p'pDDT	1,1,1-Trichloro-2,2-bis(p'p'-chlorophenyl) ethane	"	154	2
o'p' DDT	1,1,1-Trichloro-2,2-bis(o'p'-chlorophenyl) ethane	"	1756	1
Mirex	Dodecachlorooctahydro-1,3,3-metheno-2H-cyclobuta(cd)-pentalene	"	04-9057	1
Aldrin	1,2,3,4,10,10-Hexachloro-1,4-4a,5,8,8a-hexahydro-1,4-endo-5,8dimethanonapthalene	"	04-9000	1
Endrin	Hexachloroepoxyoctahydro-endo,endodimethanonapthalene	"	04-9032	1
Dieldrin	Hexachloroepoxyoctahydro-endo-exodimethanonapthalene	"	10139	1
Lindane	isomer of 1,2,3,4,5,6-hexachlorocyclohexane	"	04-9049	1
Heptachlor Epoxide	1,4,5,6,7,8-Heptachloro-ea.4.7.7a-tetrahydro-4,7-methanoindane	"	1770	1
Arochlor 1254*	A Mixture of Chlorinated Terphyenyls	PWRC	A1- 45WL	10

* Mixed as a separate standard.

APPENDIX C

RELATIVE RETENTION TIMES ON TWO GLC COLUMNS OF PESTICIDES PREVIOUSLY SEPARATED INTO FOUR FRACTIONS BY TLC (DIELDRIN= 1.00)

COMPOUND	TLC FRACTION	QF-1	OV-1
DIELDRIN	1	1.00	1.00
LINDANE	1	0.221	0.211
ALDRIN	1	0.276	0.493
HEPTACHLOR EPOXIDE	1	0.589	0.639
4,4-DICHLOROBENZOPHENONE	1	0.798	0.854
ENDRIN	1	1.166	1.394
o'p'-DDD	2	0.826	0.821
p'p'-DDD	2	1.324	1.277
o'p'-DDE	3	0.495	0.741
o'p'-DDT	3	0.905	0.998
p'p'-DDT	3	1.425	1.859
p'p'-DDE	4	0.684	0.923
MIREX	4	1.234	1.050
AROCHLOR 1254	4*		
Peak # 3		0.341	0.684
Peak # 6		0.521	0.852
Peak #10		0.912	1.524
Peak #11		0.991	1.581
Peak #13		1.254	1.916

* Some of the Arochlor 1254 was also found in Fraction #3.

APPENDIX D

RESIDUE PROFILES FOR ANALYZED EGGS

YORK RIVER

SAMPLE SITE	ALIQUOT* WEIGHT(g)	LIPID** WEIGHT(g)	EGGSHELL THICKNESS(mm)	RESIDUES IN PPM								
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	OTHER	HEPTACHLOR EPOXIDE	DCBP
CB-2(1)	18.16	0.31	0.415	7.75	12.61	0.14	20.50	31.76	3.76	-	T	T
CB-5(1)	18.48	0.45	0.420	7.84	15.31	-	23.15	19.52	1.73		T	T
CB-6(2)	27.00	0.57	0.359	7.89	7.89	2.62	18.55	23.24	0.77		T	T
CB-6(3)	21.47	0.93	0.335	9.90	8.78	3.34	22.02	36.50	1.46		T	T
CB-13(1)	20.64	0.28	0.433	3.69	2.32	0.26	6.27	6.27	0.59		T	T
CB-13(2)	19.59	0.25	0.454	4.34	4.15	0.48	8.97	6.48	0.61		T	T
CB-13(3)	18.41	0.25	0.430	5.36	4.89	0.11	10.36	15.22	0.76		T	T
CB-19(1)	21.17	0.48	0.406	3.07	3.29	-	6.36	5.02	0.46		T	T
WP-1(1)	21.55	0.82	0.319	8.93	7.97	1.94	28.84	23.57	1.46		T	T
WP-1(2)	21.00	0.73	0.394	6.48	7.79	1.55	20.60	20.60	2.35		T	T
WP-1(3)	21.35	0.86	0.376	2.84	5.64	0.59	2.72	2.72	0.94		T	T
Mean			0.395	6.19	7.33	1.00	17.35	17.35	1.35			

* ADJUSTED ALIQUOT
** LIPID OBTAINED FROM PRECEEDING ALIQUOT

MOBJACK BAY

SAMPLE SITE	WET WEIGHT	LIPID WEIGHT	SHELL THICKNESS	RESIDUES IN PPM							
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	HEPTACHLOR EPOXIDE	DCBP
M-31(1)	20.00	0.31	0.438	9.88	2.91	0.46	13.35	9.51	1.21	T	0.54
M-31(2)	20.58	0.30	0.408	6.26	7.39	0.17	13.82	25.24	0.91	T	T
M-31(3)	22.01	0.33	0.416	4.75	6.53	0.14	11.42	10.00	0.60	T	T
M-31-11(1)	21.23	0.39	0.404	3.54	6.25	0.36	9.79	8.70	0.66	T	T
WN-1(2)	25.06	0.93	0.355	3.36	5.39	0.36	9.11	7.32	0.38	T	T
WN-1(3)	24.48	0.28	0.381	4.56	5.14	0.54	10.24	12.92	0.55	T	T
WN-13(1)	21.14	0.29	0.407	5.20	4.70	-	9.90	23.99	1.86	T	T
Mean	-	-	0.401	5.36	5.47	0.29	11.09	13.95	0.88	-	-

NEW POINT COMFORT

SAMPLE SITE	WET WEIGHT	LIPID WEIGHT	SHELL THICKNESS	RESIDUES IN PPM								
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	OTHER	HEPTACHLOR EPOXIDE	DCBP
M-4(1)	24.42	-	0.364	-	5.69	-	5.69	18.97	0.81	-	T	T
M-4(2)	20.41	0.62	0.418	4.62	9.22	0.34	14.98	14.63	1.23	LINDANE 0.03	T	T
M-7(2)	24.75	0.86	0.410	5.42	6.08	0.11	12.61	15.58	0.56	-	T	T
M-7(3)	-	-	0.449	-	4.30	0.24	4.54		1.29		T	T
M-9(1)	19.72	0.99	0.488	2.27	6.61	-	8.88	3.34	1.04		T	T
M-10(1)	27.08	1.02	0.459	3.63	5.62	-	9.25	5.46	0.80		T	T
M-10(2)	22.45	0.53	0.449	7.13	6.05	0.49	13.67	14.36	0.81		T	T
M-10(3)	26.51	0.72	0.475	2.29	4.60	0.44	7.33	4.46	0.40		T	T
M-10(4)	22.13	0.37	0.384	10.04	3.70	1.01	14.75	10.85	0.63		T	T
M-10-11(1)	24.68	0.20	0.420	6.91	5.27	0.23	12.41	11.76	0.83		T	T
M-10-11(2)	24.46	0.79	0.445	3.34	3.27	0.08	6.69	9.72	1.16		T	T
M-11(1)	21.62	0.52	0.427	3.58	4.75		8.33	6.27	0.60		T	T
M-11(2)	22.25	0.83	0.428	4.82	8.10	0.18	13.10	-	0.39		T	T
M-11(3)	24.70	1.01	0.402	8.99	8.10	0.92	18.01	1.48	0.72		T	T
M-24(3)	20.03	0.30	0.459	5.18	4.70	0.08	9.96	6.85	1.04	-	T	-
M-30(1)	31.36	1.38	0.339	5.06	7.69	0.72	13.47	14.99	1.63	LINDANE 0.10	T	

NEW POINT COMFORT (CONT.)

SAMPLE SITE	WET WEIGHT	LIPID WEIGHT	SHELL THICKNESS	RESIDUES IN PPM								
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	OTHER	HEPTACHLOR EPOXIDE	DCBP
NPC-16(3)	19.78	0.41	0.415	4.12	5.83	0.42	10.47	6.83	0.73		T	T
NPC-17(3)	19.93	0.42	0.463	2.46	4.16	0.12	6.74	4.33	0.43		T	T
NPC-17-11(1)	23.10	0.25	0.507	5.61	7.08		12.69	9.03	0.95		T	T
NPC-23(1)	14.66	0.66	0.420	8.46	11.88		20.34	9.81	0.64		T	T
NPE-23-11(1)	20.59	0.45	0.428	4.26	8.05	1.26	13.56	5.34	0.26	LINDANE 0.68	T	T
NPC-25(1)	19.93	-	0.358	7.76	6.34	0.76	14.86	24.12	1.27		T	T
NPC-32(2)	20.76	0.40	0.386	0.83	4.35		5.78	1.32	0.54		T	T
NPC-32(3)	17.86	0.40	0.452	2.52	6.98	-	9.50	4.75	0.89		T	T
NPC-35(2)	25.72	0.77	0.418	4.19	4.23	0.36	9.78	11.31	0.81		T	T
NPC-35(3)	22.87	0.73	0.299	2.82	3.73	0.26	6.81	4.75	0.37		T	T
NPC-35(4)	24.25	0.38	0.395	5.52	5.30	0.03	11.10	19.86	1.09	ENDRIN 0.07	T	T
NPC-35-11(1)	21.67	-	0.368	5.53	7.71	0.40	13.74	21.70	1.64	LINDANE 0.16 ENDRIN 0.20	T	T
NPC-35-11(2)	19.48	0.81	0.382	3.66	7.63	T	10.99	7.91	2.13		T	T
NPC-40(1)	24.01	0.70	0.458	5.30	5.70		11.00	10.85	0.57		T	T
NPC-40(2)	19.90	0.47	0.461	6.80	4.78	-	11.58	13.61	0.70		T	T
NPC-40(3)	22.72	0.76	0.468	33.28	7.96	0.30	42.04	39.23	2.72		T	T

NEW POINT COMFORT (CONT.)

SAMPLE SITE	WET WEIGHT	LIPID WEIGHT	SHELL THICKNESS	RESIDUES IN PPM								
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	OTHER	HEPTACHLOR EPOXIDE	DCBP
NPC-43(1)	20.00	-	0.411	7.63	8.56		16.19	9.56	1.30		T	T
NPC-43(2)	19.82	0.35	0.475	4.95	6.65	0.35	11.95	10.90	0.95		T	T
NPC-43(3)	20.81	0.18	0.396	5.38	5.11		10.49	7.77	0.58	-	T	T
NPC-53(1)	32.69	0.55	0.516	2.08	9.03	0.23	11.26	2.34	1.32	LINDANE 0.02	T	T
NPC-53(2)	30.93	0.59	0.477	11.91	6.88	0.15	19.02	11.09	1.55	-	T	T
Mean	-	-	0.427	5.95	6.26	0.27	12.25	10.71	0.96	-	-	-

RAPPAHANNOCK RIVER

SAMPLE SITE	WET WEIGHT	LIPID WEIGHT	SHELL THICKNESS	RESIDUES IN PPM							
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	HEPTACHLOR EPOXIDE	DCBP
L-4(1)	20.65	0.22	0.445	3.91	1.18		5.09	4.27	0.61	T	-
L-4(2)	20.62	0.35	0.385	4.85	3.87		8.72	11.66	0.79	T	T
MOR-8(1)	20.38	0.56	0.459	5.04	3.88	-	8.92	10.00	0.70	T	T
U-38(1)	19.83	-	0.446	5.28	9.92	9.42	24.62	15.03	1.20	T	T
IRV-8(1)	35.67	1.01	0.317	2.96	4.14	0.09	7.19	6.27	0.42	T	T
WIL-10(1)	22.89	0.87	0.433	5.73	4.54	0.02	10.50	17.76	0.75	T	T
WIL-11(1)	22.98	0.49	0.414	4.03	4.99		9.02	9.72	1.01	T	
Mean	-	-	0.414	4.54	4.69	1.36	10.58	10.67	0.78	-	-

FLEET'S BAY

SAMPLE SITE	WET WEIGHT	LIPID WEIGHT	SHELL THICKNESS	RESIDUES IN PPM								
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	HEPTACHLOR EPOXIDE	DCBP	OTHER
FB-14(1)	23.26	0.39	0.375	4.12	4.33	0.16	8.61	8.23	0.67	T	T	-
FB-14(2)	26.37	0.70	0.329	3.95	5.51	0.24	9.70	5.13	0.81	T	T	
FB-14(3)	24.94	0.60	0.351	4.79	5.63	0.44	10.86	11.15	0.86	T	T	
FB-24-11(1)	19.34	0.77	0.456	9.70	5.65	0.23	15.58	9.71	0.93	T	T	-
FB-25(1)	19.08	0.66	0.522	9.36	9.34	0.44	19.14	15.93	3.20	0.56	T	LINDANE 0.12
FB-25(2)	35.94	0.97	0.393	3.61	7.40	0.02	11.03	4.46	2.47	T	T	
FB-28(1)	19.44	0.45	0.409	2.36	2.97	0.14	5.47	3.99	0.96	T	T	
FB-28(2)	21.72	0.36	0.448	2.63	1.80	-	4.43	3.62	0.50	T	T	
FB-28(3)	19.89	0.57	0.451	2.76	3.94	0.12	6.86	4.03	0.66	T	T	
FB-28-11(1)	21.04	0.31	0.467	1.82	3.79	-	5.61	4.25	0.85	T	T	-
FB-31(1)	24.21	0.75	0.402	6.01	8.31	0.17	14.32	12.17	0.83	T	T	LINDANE 0.04
FB-34(1)	32.18	1.18	0.500	4.39	6.54	0.59	11.43	10.74	0.61	T	T	-
FB-38(1)	20.12	0.64		3.09	3.32		6.41	5.51	1.14	T	T	
R-6(1)	24.50	-	0.402	5.02	8.69		13.71	13.71	1.32	T	T	
R-6(2)	21.64	0.74	0.411	6.09	4.48	0.36	10.93	10.93	0.94	T	T	
Mean	-	-	0.422	4.64	5.45	0.19	10.27	9.24	1.11	-	-	-

POTOMAC RIVER

SAMPLE SITE	WET WEIGHT(g)	LIPID WEIGHT(g)	SHELL(mm) THICKNESS	RESIDUES IN PPM								
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	OTHER	HEPTACHLOR EPOXIDE	DCBP
KINS- 4(1)	40.54	1.02	0.447	4.40	6.06	-	10.46	7.39	0.87	LINDANE 0.04	T	T
KINS- 7(1)	11.34	0.49	0.466	4.07	21.54	1.16	26.77	14.73	3.56	LINDANE 0.08	0.84	1.21
KINS- 9(1)	24.83	0.81	0.399	3.75	8.19	0.06	11.00	13.02	1.69	-	T	T
KINS-11(1)	22.31	0.65	0.380	6.31	10.28	0.80	17.39	19.86	3.09	ENDRIN 0.90	0.67	0.60
Mean	-	-	0.423	4.63	11.51	0.51	13.91	14.00	2.30	-	-	-

OCEAN

SAMPLE SITE	WET WEIGHT	LIPID WEIGHT	SHELL THICKNESS	RESIDUES IN PPM								
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	OTHER	HEPTACHLOR EPOXIDE	DCBP
CC-3(1)	23.96	0.20	0.434	5.86	8.33		14.19	7.02	1.22		T	T
CC-4(1)	20.63	0.57	0.449	6.30	2.19	T	8.49	12.86	0.28		T	T
T-9(1)	21.51	0.21	0.386	6.05	3.94	0.36	10.35	12.33	1.14		T	T
T-9(2)	20.63	0.77	0.383	1.06	1.00	-	2.06	-	-		T	T
T-31(1)	21.95	0.20	0.463	6.16	6.33	0.73	13.26	9.03	0.71	-	T	T
CW-3(1)	22.60	0.35	0.408	14.75	14.72	1.29	30.76	1.53	2.82	LINDANE 0.08	0.82	T
WACH-4(1)	21.12	-	0.379	6.36	5.44	0.66	12.46	12.29	0.77	-	T	T
Mean	-	-	0.415	6.65	5.99	0.43	13.08	9.86	0.99	-	-	-

BAY

SAMPLE SITE	WET WEIGHT	LIPID WEIGHT	SHELL THICKNESS	RESIDUES IN PPM								
				DDE	DDD	DDT	TOTAL DDT	PCB	DIELDRIN	OTHER	HEPTACHLOR EPOXIDE	DCBP
F-14(1)	36.94	1.22	0.486	5.10	10.35		15.45	5.47	0.75		T	T
P-2(1)	29.39	0.58	0.446	4.11	5.28	0.16	9.55	13.03	0.79	-	T	T
P-2(2)	20.01	0.34	0.408	5.17	5.42	-	10.59	5.36	1.03	ENDRIN 0.28 LINDANE0.03	T	T
Mean	-	-	0.447	4.79	7.01	0.05	11.86	7.95	0.86	-	-	-

APPENDIX E

CHANGES IN EGGSHELL PARAMETERS BETWEEN FIRST AND SECOND CLUTCH

SAMPLE SITE	EGGSHELL THICKNESS				EGGSHELL WEIGHT				THICKNESS INDEX			
	FIRST CLUTCH		SECOND CLUTCH		FIRST CLUTCH		SECOND CLUTCH		FIRST CLUTCH		SECOND CLUTCH	
	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN
CB-6	3	0.354	1	0.367	3	4.67	1	4.63	3	1.81	1	1.88
M-31	3	0.421	1	0.404	3	6.06	1	5.41	3	2.13	1	2.08
NPC-17	3	0.486	1	5.07	1	6.00	0	-	1	2.30	0	-
NPC-23	3	0.442	2	0.434	3	6.08	2	5.57	3	2.12	2	2.04
NPC-35	4	0.385	3	0.381	3	5.37	2	5.70	3	1.92	2	2.02
M-10	4	0.442	2	0.432	4	5.96	1	5.49	4	2.13	1	2.07
FB-24	3	0.469	1	0.456	2	6.41	1	6.11	2	2.37	1	2.32
FB-28	3	0.436	2	0.429	3	7.26	1	7.24	3	2.22	1	2.35

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VITAE

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